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STRUCTURE DES POPULATIONS DE SÉBASTE DE L'ATLANTIQUE DU NORD-OUEST DANS UN CONTEXTE DE GESTION DES STOCKS ET D'ÉVOLUTION

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ALEXANDRA VALENTIN

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À Jean-François,

à ma famille

et à Wilson et Baxter

pour tout ce qu'ils représentent

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Le corps de la thèse est constitué de trois chapitres (chapitres 2, 3 et 4) rédigés sous forme d'articles destinés à être publiés dans des revues scientifiques. Les références pour ces articles sont les suivantes :

Chapitre 2 :

Valentin, A. E., X. Penin, J.-P. Chanut, J.-M. Sévigny, & F. J. Rohlf. (Accepté). Arching effect affecting fish body shape in geometric morphometric studies. *Journal of Fish Biology*.

Chapitre 3 :

Valentin, A. E., J.-P. Chanut, X. Penin, & J.-M. Sévigny. (À soumettre). Combining microsatellites and geometric morphometrics for redfish species discrimination in the northwest Atlantic: methodological considerations and new insights on introgressive hybridization.

Chapitre 4 :

Valentin, A. E., J.-M. Sévigny, J.-P. Chanut & X. Penin. (À soumettre). Combining microsatellites and geometric morphometrics for the study of redfish population structure in the northwest Atlantic.

RÉSUMÉ

Une approche multidisciplinaire combinant génétique (13 microsatellites) et morphométrie géométrique (forme corporelle) a été appliquée à l'étude des trois espèces de sébaste (*Sebastes* sp.) du nord-ouest Atlantique, pour fournir de l'information utile dans un contexte de gestion des pêches et d'évolution. Le premier objectif consistait à documenter la structure des populations de *Sebastes mentella* et de *S. fasciatus*, les deux espèces d'importance commerciale. Le deuxième objectif visait à étudier plus avant le processus d'hybridation introgressive, préalablement documenté entre ces deux espèces. Un échantillon limité de *S. marinus* n'a pas permis de déterminer la structure de populations pour cette espèce, mais a suffi pour évaluer sa participation au processus d'hybridation introgressive. Sur le plan méthodologique, l'étude visait à documenter la puissance, les limites et la complémentarité des approches génétique et morphométrique utilisées pour la discrimination des espèces et des populations.

Au niveau des populations, les approches génétique et morphométrique convergent pour décrire la structure des populations de *S. fasciatus* et de *S. mentella*. Pour les deux espèces, la structure est faible, avec une hétérogénéité locale superposée à une structure à plus grande échelle. La structure à grande échelle appuie l'hypothèse du *member-vagrant* qui postule que les facteurs environnementaux sont déterminants pour structurer les populations.

Pour *S. mentella*, les résultats suggèrent la présence d'une seule population dans le golfe du Saint-Laurent et le chenal Laurentien (GSL–LCH). Le flux génique entre le Groenland et le nord des Grands Bancs de Terre-Neuve (TN) est suffisant pour entraîner l'absence de différences génétiques entre ces régions, mais la morphométrie suggère que ces échanges sont quand même limités. Le Labrador pourrait abriter une population isolée, alors que les sébastes du fjord du Saguenay constitueraient une population puits.

Pour *S. fasciatus*, les résultats suggèrent la présence de trois populations très faiblement différenciées à grande échelle : autour des Grands Bancs de TN, dans le golfe du Maine et dans le GSL–LCH. Il existe toutefois de l'hétérogénéité génétique dans le GSL–LCH, dont l'origine n'a pas été identifiée. Le plateau de la Nouvelle-Écosse (NÉ) semble abriter une population différente de celle de la pente du plateau de la NÉ, qui s'apparente elle-même à la population des Grands Bancs de TN. La population du golfe du Maine se démarque de celle du plateau de la NÉ : les courants coulant du plateau de la NÉ vers le golfe du Maine n'encourageraient donc pas la dérive larvaire. Par contraste, la présence de quelques individus génétiquement introgressés dans le golfe du Maine indique la possibilité d'immigration à partir du GSL–LCH. Le fjord de Bonne Bay abrite une population isolée qui découle probablement d'un événement fondateur, suivi d'un flux génique limité avec l'extérieur.

La structure des populations observée dans la zone de sympatrie n'est pas identique pour les deux espèces, ce qui complique les stratégies de gestion qui peuvent, dans la pratique, difficilement considérer les deux espèces séparément. Les enjeux pour la gestion sont discutés en tenant compte de la contribution relative des espèces à la pêche.

Les données génétiques confirment la présence d'hybridation introgressive asymétrique entre *S. fasciatus* et *S. mentella*. Le processus est restreint au GSL–LCH : il contribue à la structure des populations à large échelle et à l'hétérogénéité locale. Les résultats suggèrent également une participation de *S. marinus* au processus d'hybridation introgressive.

Les approches génétique et morphométrique permettent toutes deux de discriminer significativement les espèces. La corrélation significative observée entre les deux jeux de données confirme l'hypothèse d'une forte détermination génétique de la forme des sébastes. Les résultats réfutent toutefois l'hypothèse d'une convergence morphologique consécutive à l'hybridation introgressive. La population puits du fjord du Saguenay, où les conditions environnementales sont très différentes de celles du GSL, semble démontrer la présence d'un certain niveau de plasticité de la forme du corps.

Sur le plan méthodologique, il est possible de classifier les individus par espèce, sans assignation *a priori*, aussi bien par l'approche génétique que par l'approche morphométrique. La morphométrie nécessite une calibration préalable du modèle discriminant, à l'aide de données génétiques.

Une autre étape méthodologique importante est l'élimination de la variabilité morphométrique générée par la posture du poisson lors de la capture des données. Cet artefact est indépendant du mode de préservation. Il se corrige efficacement par projection des données morphométriques orthogonalement à un vecteur qui modélise la déformation associée à l'artefact.

Au niveau génétique, l'utilisation de microsatellites supplémentaires améliore la discrimination interspécifique. Tous les loci permettent de discriminer significativement les espèces, sur la base de l'indice de différenciation génétique F_{ST}. Les valeurs de F_{ST} sont toutefois contraintes par le polymorphisme des loci, ce qui rend l'indice de différenciation G'_{ST} (standardisé pour l'effet du polymorphisme) nécessaire pour comparer les niveaux de différenciation exprimés par les différents loci. Les loci les plus discriminants présentent un motif microsatellite interrompu. Il est proposé que le niveau d'homoplasie pourrait différer entre les loci.

TABLE DES MATIÈRES

LISTE DES TABLEAUX

CHAPITRE 2 : ARCHING EFFECT AFFECTING FISH BODY SHAPE IN GEOMETRIC MORPHOMETRIC STUDIES

CHAPITRE 3 : COMBINING MICROSATELLITES AND GEOMETRIC MORPHOMETRICS FOR REDFISH SPECIES DISCRIMINATION IN THE NORTHWEST ATLANTIC : METHODOLOGICAL CONSIDERATIONS AND NEW INSIGHTS ON INTROGRESSIVE HYBRIDIZATION

LISTE DES FIGURES

CHAPITRE 1 : INTRODUCTION GÉNÉRALE

CHAPITRE 2 : ARCHING EFFECT AFFECTING FISH BODY SHAPE IN GEOMETRIC MORPHOMETRIC STUDIES

CHAPITRE 1

INTRODUCTION GÉNÉRALE

1.1 CONTEXTE DE L'ÉTUDE

Le sébaste est un poisson marin d'importance commerciale dans le nord-ouest de l'Atlantique. On y reconnaît trois espèces : *Sebastes marinus* (Linné, 1758), *S. mentella* (Travin, 1951) et *S. fasciatus* (Storer, 1856). La pêche commerciale repose majoritairement sur les espèces *S. mentella* et *S. fasciatus*. Durant les vingt dernières années, aucun recrutement significatif n'a été observé et la taille des stocks commerciaux de sébaste a dramatiquement baissé. En conséquence, les quotas n'ont cessé de diminuer et la pêche est interdite dans le golfe du Saint-Laurent depuis 1995. Cette situation a fait naître la nécessité d'étudier plus profondément la structure des populations. Dans ce contexte, le programme de recherche multidisciplinaire sur le sébaste (1996-1999) du ministère des Pêches et des Océans du Canada a permis de décrire la distribution des deux espèces et a conduit à plusieurs constats. Il est apparu que les populations exploitées sont souvent composées d'un mélange de *S. mentella* et de *S. fasciatus*. Il est également ressorti qu'il existe un phénomène d'hybridation introgressive entre les deux espèces (c.-à-d. une hybridation suivie d'un croisement des hybrides avec les espèces parentales), dans le golfe du Saint-Laurent et le chenal Laurentien (Roques *et al.*, 2001; Gascon, 2003; Valentin *et al.*, 2006).

Plusieurs questions restent cependant en suspens (DFO, 2001*a*, 2001*b*; CCRH, 2003). Dans un contexte de gestion de la ressource, on s'interroge notamment sur la pertinence biologique des zones de gestion (Figure 1.1) et, en particulier, sur (1) la validité de la délimitation entre l'Unité 1 (golfe du Saint-Laurent) qui est actuellement sous

moratoire et l'Unité 2 (sud de Terre-Neuve) qui continue à soutenir la pêche, (2) la relation entre les populations nouvellement exploitées de la zone de pêche 1F (sud-ouest du Groenland) et les stocks des eaux canadiennes adjacentes (mer du Labrador) et (3) la relation entre l'Unité 3 (plateau néo-écossais et golfe du Maine canadien) et le stock américain du golfe du Maine où un important recrutement d'origine inconnue a récemment été observé (Northeast Fisheries Science Center, 2001). D'un point de vue évolutif, les principales interrogations portent sur les facteurs responsables de l'hybridation introgressive et sur les conséquences du phénomène pour l'intégrité des espèces et la structure des populations.

1.2 CIRCULATION GÉNÉRALE DANS LE NORD-OUEST ATLANTIQUE

Bien que l'aire de distribution des sébastes dans l'Atlantique du nord-ouest soit très étendue (Figure 1.1), elle est caractérisée par une continuité environnementale remarquable, tel qu'en témoigne le patron général de circulation des eaux (Sutcliffe *et al.*, 1976). Le courant du Labrador, qui s'écoule vers le sud le long de la côte du même nom, est caractérisé par deux composantes. La première, située sur le plateau continental, contient un mélange d'eau originaire du détroit d'Hudson et du courant de Baffin. La deuxième composante, qui circule plus au large au niveau de la pente du plateau continental, possède les caractéristiques des eaux du courant du Groenland. Plus au sud, au large de Terre-Neuve, les deux branches du courant du Labrador se séparent. La branche du large

contourne les Grands Bancs et, à la hauteur de 48° N, une partie du courant dévie vers l'est pour rejoindre la circulation anticyclonique caractéristique du Bonnet Flamand (Templeman, 1976). La branche côtière du courant du Labrador contourne la péninsule d'Avalon en direction de l'ouest en perdant ses caractéristiques arctiques. L'eau entre dans le golfe du Saint-Laurent par le détroit de Cabot en longeant la côte de Terre-Neuve. Des échanges occasionnels entre les eaux du golfe du Saint-Laurent et celles du courant du Labrador ont lieu au niveau du détroit de Belle-Isle. La majorité de l'eau circule dans le golfe en suivant un parcours cyclonique. Elle rejoint le courant de Gaspé, puis s'écoule plus lentement sur le plateau madelinien. Elle ressort finalement par le détroit de Cabot, en longeant le Cap Breton, et atteint le plateau néo-écossais où elle se mélange avec l'eau de la pente du plateau continental. L'eau du plateau néo-écossais entre alors dans le golfe du Maine par le courant de la Nouvelle-Écosse qui contourne Cap Sable. Le golfe du Maine est caractérisé par la présence d'une gyre cyclonique sur le bassin central, dont l'intensité est maximale en mai. Le temps de résidence moyen de l'eau de surface est d'une année, comparativement à un an et demi pour l'eau profonde. Le principal apport d'eau profonde a lieu par le chenal du nord-est, depuis la pente du plateau continental (Brown & Beardsley, 1978; Conkling, 1995).

La grande continuité de l'environnement marin est en général peu favorable à la différenciation des populations, car il existe peu de barrières à la dérive larvaire et à la migration. Cette tendance a été confirmée par de nombreuses études génétiques (Ward *et*

al., 1994; Shaklee & Bentzen, 1998; Ward, 2000). Cependant, il a également été montré que des caractéristiques environnementales à plus petite échelle (ex. gyres, tourbillons, etc.), couplées au comportement des individus (ex. migrations verticales), peuvent favoriser la rétention larvaire et ainsi contribuer à fragmenter les populations marines (Palumbi 1999; Largier, 2003).

1.3 ORIGINE ET TAXONOMIE DES ESPÈCES DE SÉBASTE DE L'ATLANTIQUE

Le genre *Sebastes* est représenté par une centaine d'espèces dans le Pacifique, alors que seulement quatre espèces sont dénombrées dans l'Atlantique nord. Ces dernières semblent descendre d'une espèce qui aurait colonisé l'Atlantique nord à partir du Pacifique, en passant par le détroit de Béring et l'Arctique, vers la fin de l'ère tertiaire. Divers événements de spéciation auraient alors conduit à la formation des quatre espèces actuelles *S. marinus*, *S. mentella*, *S. fasciatus* et *S. viviparus* (Kendall, 1991). *Sebastes marinus* et *S. mentella*, sont des espèces d'eaux profondes distribuées à l'échelle de l'Atlantique nord. *Sebastes fasciatus* et *S. viviparus* sont des espèces plus côtières et de moindre profondeur. *Sebastes fasciatus* est restreinte à l'ouest du bassin nord-atlantique, alors que *S. viviparus* est retrouvée dans le centre et à l'est. *Sebastes viviparus* ne sera pas considérée dans la présente étude qui se concentre essentiellement sur l'Atlantique du nord-ouest.

Historiquement, on pensait que les populations de sébaste de l'Atlantique du nord-ouest n'étaient composées que d'une seule espèce. Les premières études morphologiques ont révélé la présence de trois espèces. En 1957, Templeman et Sandeman sont les premiers à reconnaître deux types de sébaste qu'ils définissent comme *S. marinus* (Linné, 1758) et *S. mentella* (Travin, 1951). En 1968, Barsukov décrit une troisième espèce qu'il identifie comme étant *S. fasciatus* (Storer, 1856). En 1974, Litvinenko caractérise, dans les eaux côtières peu profondes du golfe du Maine, une sous-espèce de *S. fasciatus*. Elle est nommée *S. fasciatus kellyi* en l'honneur de George Kelly qui l'avait précédemment observée (Kelly & Barker, 1961).

1.4 DISTRIBUTION DES ESPÈCES DANS LE NORD-OUEST ATLANTIQUE

Sebastes marinus est relativement peu fréquente dans le nord-ouest Atlantique, sauf dans la région du Bonnet Flamand (DFO, 2001*a*). Pour cette raison, la présente étude se concentrera sur *S. mentella* et *S. fasciatus*, les espèces les plus abondantes qui soutiennent la pêche. Ces deux espèces sont distribuées selon un gradient nord-sud. *Sebastes mentella* domine dans la baie de Baffin et les eaux du Labrador, tandis que *S. fasciatus* domine dans le golfe du Maine et sur le plateau néo-écossais. Les aires de distribution des deux espèces se chevauchent dans la région de Terre-Neuve, sur la pente du plateau néo-écossais et dans le golfe du Saint-Laurent (Ni, 1982; Atkinson, 1987; Rubec *et al.*, 1991; Gascon, 2003; Valentin *et al.*, 2006). Les sébastes sont considérés comme des poissons de fond et, en règle

générale, *S. mentella* privilégie les zones plus profondes (≥ 300 m) que *S. fasciatus* (≤ 300 m). Cependant, les prises réalisées avec des chaluts semi-pélagiques indiquent que le sébaste est également présent plus haut dans la colonne d'eau. Par ailleurs, il a été observé que la distribution verticale du sébaste varie de façon diurne et saisonnière (Gauthier & Rose, 2002). Ainsi, dans les zones de sympatrie (c.-à-d. les régions où les deux espèces cohabitent), la bathymétrie ne constitue pas une barrière étanche entre les deux espèces. Finalement, les sébastes tendent à être distribués sous forme d'agrégations. Les relevés de pêche annuels suggèrent la présence récurrente de grandes agrégations dans certaines régions, à une date donnée, mais montrent également que les agrégations se déplacent au cours de l'année (Power, 2003).

1.5 CRITÈRES USUELS D'IDENTIFICATION DES ESPÈCES

Trois critères sont communément utilisés pour différencier les deux principales espèces commerciales de sébaste dans le nord-ouest de l'Atlantique. Il s'agit (i) du nombre de rayons mous à la nageoire anale (AFC : ≥ 8 pour *S. mentella*, ≤ 7 pour *S. fasciatus*; Ni 1981*a*, 1982), (ii) du passage intercostal du muscle de la vessie natatoire (EGM : entre les côtes 2 et 3 pour *S. mentella*, entre les côtes 3 et 4 ou + pour *S. fasciatus*; Ni 1981*a*, 1981*b*) et (iii) du génotype au locus de la malate déshydrogénase (*MDH-A**). Le locus *MDH-A** est caractérisé par deux allèles co-dominants dont la combinaison produit trois génotypes possibles. *MDH-A*11* caractérise *S. mentella*, alors que *MDH-A*22* est associé à *S.*

fasciatus (Payne & Ni 1982; McGlade *et al.* 1983). Les spécimens hétérozygotes *MDH-A*12* ne peuvent pas être assignés à une espèce, et sont considérés comme étant d'origine hybride. En 1991, Rubec *et al.* ont observé la présence significative du génotype hétérozygote *MDH-A*12* dans le golfe du Saint-Laurent (Unité 1). La comparaison des patrons d'AFC, d'EGM et de MDH a mis en évidence un manque de concordance de ces critères, ce qui indiquait la présence de caractéristiques propre à *S. mentella* chez *S. fasciatus* et réciproquement. Pour cette raison, Rubec *et al.* (1991) ont émis l'hypothèse de la présence d'un phénomène d'hybridation introgressive dans le golfe du Saint-Laurent. Des études génétiques portant sur l'ADN ribosomal (ADNr) et l'ADN microsatellite ont confirmé cette hypothèse (Desrosiers *et al.*, 1999; Roques *et al.*, 2001).

1.6 REPRODUCTION ET RECRUTEMENT

Les espèces de sébaste de l'Atlantique du nord-ouest sont lécithotrophiques vivipares. En règle générale, la copulation a lieu à l'automne (Ni & Templeman, 1985). C'est donc à cette période de l'année que la composition génétique des populations est déterminée. La copulation s'accompagne probablement d'un comportement de cour et de choix du partenaire (Helvey, 1982; Shinomiya & Ezaki, 1991). Les femelles peuvent s'accoupler avec plus d'un mâle et stockent le sperme jusqu'à la fertilisation interne qui se produit vers la fin de l'hiver. Les larves sont relâchées au printemps, près de la surface. Bien qu'elles soient capables de nager, elles sont encore fortement tributaires des courants

qui jouent un rôle déterminant dans leur distribution. Ensuite, les larves se métamorphosent progressivement en juvéniles. Au début de l'automne, les juvéniles migrent vers le fond où ils continuent leur développement vers le stade adulte et passent la majorité de leur vie d'adulte (Kenchington, 1984). Le synchronisme du cycle reproducteur varie entre les populations de la même espèce, en fonction de la région considérée (Saborido-Rey, 1994; St-Pierre & de Lafontaine, 1995), ce qui contribue à la différenciation des populations. Au niveau des espèces, il a été observé que le synchronisme du cycle reproducteur de *S. mentella* et de *S. fasciatus* est élevé dans le golfe du St. Laurent (St-Pierre & de Lafontaine, 1995), ce qui favoriserait l'occurrence d'hybridation introgressive entre les deux espèces (Roques *et al.*, 2001).

Bien qu'une nouvelle cohorte (c.-à-d. une classes d'âge) soit produite annuellement, le recrutement est extrêmement variable d'une année à l'autre. En général, les pics de recrutement sont observés tous les cinq à douze ans (DFO, 2001*a*). À plusieurs occasions (c.-à-d., en 1966, 1974, 1985 et 1988), et tout particulièrement dans l'Unité 1, des classes d'âge pourtant très abondantes ont disparu avant de contribuer à la population adulte. Les raisons de ces disparitions sont encore inconnues, mais il a été déterminé que la classe d'âge de 1988 était dominée par *S. fasciatus* (Gascon, 2003; Morin *et al.* 2004).

1.7 DÉFINITION DES STOCKS DE PÊCHE

La préoccupation principale de la gestion des pêches est d'optimiser les captures, tout en favorisant la durabilité de la ressource. Dans ce contexte, l'identification de stocks constitue un enjeu majeur. La tâche est loin d'être évidente, car le concept même de « stock » est variable, selon les préoccupations des gestionnaires et des biologistes. Pour tenir compte de la structure intraspécifique de l'espèce exploitée, plusieurs définitions basées sur des critères biologiques ont été proposées. La définition centrale de « stock biologique » implique un groupe d'individus de la même espèce qui se reproduisent aléatoirement et dont l'intégrité spatio-temporelle est maintenue (Ihssen *et al.*, 1981; Carvalho & Hauser, 1994). Il existe plusieurs approches pour identifier un tel groupe. Par exemple, un stock biologique peut être déterminé en fonction des caractéristiques génétiques des populations (Ward, 2000). Le concept de « stock génétique » a été popularisé par le développement de nombreuses méthodes d'analyses moléculaires qui permettent d'identifier des unités de population isolées en terme de reproduction (Carvalho & Hauser, 1994; Park & Moran, 1997; O'Connel & Wright, 1997; Ward, 2000). Cette approche est privilégiée lorsque l'enjeu est le maintien, à long terme, de la diversité génétique de l'espèce exploitée.

Dans l'ensemble, les marqueurs génétiques indiquent que les populations marines tendent à être faiblement différenciées. Cette tendance s'explique généralement par l'effet combiné d'une grande taille efficace des populations et de l'absence de barrières à la

migration et au flux génique (Ward *et al.*, 1994; Shaklee & Bentzen, 1998; Ward, 2000). Dans ces conditions, les modèles théoriques suggèrent que, en absence de sélection, quelques migrants par génération suffiraient à homogénéiser les fréquences alléliques entre deux populations. L'échange de ces quelques migrants a des conséquences importantes pour le maintien de la diversité génétique, mais ne permet pas pour autant de considérer ces deux populations comme biologiquement homogènes et interdépendantes en terme de recrutement (Waples, 1998; Neigel, 2002). D'ailleurs ces populations auront tendance à présenter des différences au niveau de la morphologie, du comportement, de la charge parasitaire, ou de la composition élémentaire des otolithes. Pour cette raison, l'étude de ces caractéristiques constitue une alternative ou un complément approprié aux analyses génétiques pour la discrimination des stocks dans un contexte de gestion des pêches (Deriso & Quinn, 1998; Grant *et al.*, 1999, Cadrin, 2000).

Dans le cas du sébaste, l'étude de la forme corporelle par la morphométrie apparaît comme une approche intéressante pour complémenter les analyses génétiques, dans un contexte d'identification des stocks. La forme est le résultat d'un programme génétique modulé par l'environnement et la sélection naturelle (Alberch *et al.* 1979; Swain & Foote, 1999 et références incluses). Le sébaste est caractérisé par une grande longévité et il est itéropare (c.-à-d., il se reproduit plusieurs fois au cours de sa vie). Chez cette catégorie d'espèces, la forme corporelle est généralement soumise à un fort contrôle génétique : la forme des individus est peu affectée par les variations environnementales rencontrées à l'échelle d'une vie (Schultz, 1989). Au fil des générations, des populations soumises à

différentes gammes de conditions environnementales tendront néanmoins à accumuler des différences de forme moyenne, un processus appelé sélection adaptative (Alberch *et al.* 1979; Swain & Foote, 1999 et références incluses).

Pour le sébaste de l'Atlantique du nord-ouest, les études disponibles suggèrent que la forme corporelle constitue un critère approprié pour différencier à la fois les espèces (Kenchington, 1986; Misra & Ni, 1983; Saborido-Rey, 1994; Valentin, 1999; Valentin *et al.*, 2002) et les populations (Saborido-Rey 1994; Saborido-Rey & Nedreaas, 1998). De plus, les récentes études génétiques utilisant les microsatellites (Roques *et al.*, 2001) tendent à confirmer la structure des populations mise en évidence par la morphométrie, sur le banc de Saint-Pierre, les Grands Bancs et le Bonnet Flamand (Saborido-Rey, 1994).

1.8 OUTILS POUR LA DISCRIMINATION DES POPULATIONS

1.8.1 Morphométrie

Un des problèmes de base en morphométrie provient de l'hétérogénéité de taille entre les spécimens (Rohlf & Bookstein, 1987). En effet, les différences de taille ont tendance à masquer les différences morphométriques plus subtiles. De plus, des variations de taille peuvent être assorties de variations de forme. Ce phénomène est appelé allométrie. Pour ces deux raisons, la taille doit être soustraite des données morphométriques tout en

évaluant son influence sur la forme. Dans le cas du sébaste de l'Atlantique du nord-ouest, l'allométrie est limitée chez les adultes, ce qui facilite la comparaison morphométrique d'individus adultes de taille différente (Valentin, 1999).

Historiquement, les mesures morphométriques étaient prises au moyen d'un vernier et comparées deux à deux sous forme de rapport. Dans les années 1980, la définition de la forme à l'aide d'un réseau de droites (Strauss & Bookstein, 1982; McGlade & Boulding, 1986) couplée à des statistiques multivariées a permis de décrire et de comparer les formes d'une manière plus systématique. Cette approche a été concluante pour la discrimination d'espèces (Strauss, 1986; Creech, 1992) et de populations (Winans, 1984; Roby *et al.*, 1991).

Dans les années 1990, le développement de la théorie des formes et de nouvelles procédures numériques a mené à l'émergence de la morphométrie géométrique, une discipline qui est depuis en constante évolution (Marcus & Corti, 1996; Adams *et al.*, 2004). La grande innovation de cette approche est de définir et de comparer les formes à l'aide de points de repères représentant des caractéristiques anatomiques homologues entre les individus. La morphométrie géométrique est plus efficace que la morphométrie traditionnelle pour définir la forme, ce qui augmente la puissance des procédures statistiques utilisées pour tester les différences de forme (Rohlf & Marcus, 1993).

La morphométrie géométrique a permis de discriminer *S. mentella*, *S. fasciatus* et leurs hybrides, tels que définis par le génotype au locus *MDH-A**, dans le golfe du Saint-Laurent (Valentin *et al.*, 2002). À ce jour, cette approche n'a encore jamais été utilisée pour la discrimination des populations de sébaste de l'Atlantique nord-ouest. Une récente étude menée sur les populations de l'est de l'Atlantique a toutefois confirmé l'utilité de la méthode pour la discrimination des populations de sébaste (Anonymous, 2004).

1.8.2 Microsatellites

Les microsatellites sont des courtes séquences d'ADN composées de 1 à 6 paires de base (pb) répétées en tandem. Ces séquences sont dispersées dans le génome et varient en nombre et en longueur. Les microsatellites sont caractérisés par un important polymorphisme (plusieurs allèles possibles pour un locus donné), la codominance (expression équivalente des deux allèles parentaux), la transmission mendélienne et, à moins d'être situés près de gènes soumis à sélection, la neutralité (pas d'avantage sélectif). Ces caractéristiques en font des marqueurs privilégiés en génétique des populations, et tout particulièrement pour l'étude des populations à faible structure génétique. Dans le cas du sébaste de l'Atlantique du nord-ouest, les microsatellites ont permis de décrire plus finement la structure génétique des populations et de caractériser la dynamique de l'hybridation introgressive entre *S. fasciatus* et *S. mentella* (Roques *et al.*, 2001; voir paragraphes 1.10 et 1.11).

Les progrès réalisés au niveau des techniques de laboratoire ont contribué à la popularité des microsatellites, en particulier la méthode de polymérisation en chaîne de l'ADN (PCR) avec marquage fluorescent, suivie d'une électrophorèse sur séquenceur automatisé.

Parallèlement, plusieurs développements ont été réalisés dans le domaine statistique pour analyser spécifiquement les données de microsatellites. De nouveaux indices de différenciation génétique ont été proposés comme alternative aux indices traditionnellement calculés sur la base des fréquences alléliques (Goldstein & Pollock, 1997; Balloux & Lugon-Moulin, 2002). Les indices spécifiques aux microsatellites tiennent compte des différences de taille entre les allèles, ce qui permet de prendre en considération le processus mutationnel particulier aux microsatellites. Ce processus implique des changements d'une forme allélique vers l'autre par additions/soustractions successives d'une répétition du motif microsatellite.

La nécessité d'analyser des jeux de données combinant plusieurs variables très polymorphes a favorisé le développement d'analyses statistiques faisant appel à des simulations basées sur les modèles de maximum de vraisemblance, les statistiques bayésiennes et les méthodes de Monte Carlo par chaîne de Markov (Luikart & England, 1999; Sunnucks, 2000).

En outre, plusieurs études ont souligné les différents problèmes associés spécifiquement à l'analyse et à l'interprétation des données de microsatellites. Par exemple, il est maintenant reconnu que des problèmes au cours de la PCR (non-amplification, bégaiement) provoquent des erreurs dans la détermination des allèles (Bonin *et al.*, 2004; Hoffman & Amos, 2005).

Les propriétés particulières des microsatellites, eu égard au polymorphisme et au processus de mutation, ont également entraîné une réflexion sur la validité des plans d'échantillonnage (nombre d'individus par rapport au nombre et au polymorphisme des microsatellites), la précision des estimateurs statistiques et la puissance des tests statistiques (ex., Ruzzante, 1998; Hedrick, 1999; Balloux & Lugon-Moulin, 2002; Kalinowski, 2005).

1.9 IMPORTANCE DES AGRÉGATIONS

Les récentes études utilisant des marqueurs microsatellites (Roques *et al.*, 2001; Roques *et al.*, 2002) ont permis une première description de la structure des populations de sébastes de l'Atlantique du nord-ouest. Cependant, ces études comprenaient plusieurs limites. En effet, les spécimens n'étaient pas choisis au hasard, mais étaient plutôt sélectionnés sur la base de la congruence des critères usuels d'identification d'espèce. De plus, les échantillons étaient composés d'individus dispersés plutôt que d'individus provenant des grandes agrégations qui soutiennent la pêche. Cette stratégie

d'échantillonnage peut avoir sous-estimé la variabilité génétique et ignoré de l'information sur la structure génétique des populations exploitées. Dans ce contexte, une stratégie d'échantillonnage visant les grandes agrégations qui soutiennent la pêche constitue une approche plus appropriée pour étudier la structure des populations. La comparaison des agrégations à l'intérieur des unités de gestion et entre celles-ci permet de documenter la variabilité intra et inter zone sans nécessiter une sélection préalable des individus. Cette approche est recommandée pour optimiser l'identification des unités de gestion par extrapolation de données génétiques (Paetkau, 1999).

La structure des agrégations de sébaste n'a encore jamais été strictement étudiée. Cependant, Roques *et al.* (2001) ont suggéré la présence de différences génétiques à petite échelle entre plusieurs échantillons de *S. mentella*, dans le golfe du Saint-Laurent et le chenal Laurentien, en absence de différenciation significative entre les deux unités. Par ailleurs, les données de MDH suggèrent que les sébastes pris dans un même trait de chalut tendent à appartenir à la même espèce, et que les hybrides se tiennent avec *S. mentella* (Rubec *et al.*, 1991; Valentin *et al.*, 2006). L'association entre *S. mentella* et les hybrides est une caractéristique qui semble se maintenir tout au long du cycle de vie, puisqu'elle est également observée pour les larves et les juvéniles (Sévigny & de Lafontaine, 1992; Sévigny *et al.*, 2000). En plus de s'associer avec *S. mentella*, les hybrides tendent également à leur ressembler pour plusieurs caractéristiques comme les critères méristiques (AFC, EGM) et la forme corporelle (Valentin *et al.*, 2002). Ainsi, les sébastes tendent à former des agrégations d'individus qui présentent souvent des caractéristiques identiques.

1.10 STRUCTURE DES UNITÉS DE GESTION ACTUELLES

Les pêcheurs canadiens exploitent le sébaste depuis la fin des années 1940. Comme les trois espèces sont d'apparence très semblables, elles ne sont pas différenciées dans la pêche et sont gérées ensemble. À l'heure actuelle, il existe 8 unités de gestion du sébaste dans l'Atlantique du nord-ouest, dont certaines regroupent plusieurs divisions. Les unités sont : 2GHJ-3K (au nord), 3LN et 3O (Grands Bancs de Terre-Neuve), 3M (Bonnet Flamand), Unité 1 (golfe du Saint-Laurent), Unité 2 (chenal Laurentien), Unité 3 (plateau néo-écossais) et la sous-zone 5 (golfe du Maine) (Figure 1.1).

Les marqueurs génétiques de type microsatellite révèlent que les stocks de sébastes de l'Atlantique du nord-ouest sont caractérisés par une faible structure génétique (Roques *et al.*, 2001; Roques *et al.*, 2002). Ceci indique que les différentes unités de gestion sont reliées, c'est-à-dire qu'il y a échange d'individus entre les unités. Il faut cependant noter que les conséquences démographiques des échanges sont difficiles à évaluer sur la base des seules données génétiques (Waples, 1998).

Dans le nord, les récentes études utilisant les marqueurs microsatellites suggèrent la présence d'une seule grande population de *S. mentella* depuis la mer du Labrador jusqu'aux Îles Faroe (Roques *et al.*, 2002). D'autres études montrent que la mer d'Irminger, située à l'est du Groenland, est la zone d'extrusion larvaire qui alimente les populations de l'ouest du Groenland jusqu'à l'Islande. Par ailleurs, il est proposé qu'une fraction des larves dérive

depuis l'ouest du Groenland jusque dans la mer du Labrador (Saborido-Rey *et al.*, 2001). Actuellement, les sébastes de la mer du Labrador sont gérés comme un seul stock (2GHJ-3K) par le Canada, alors que les eaux situées plus à l'est sont sous la gestion de l'Union Européenne. En 2000, des prises substantielles ($>10^4$ tonnes) ont été réalisées à l'ouest du Groenland, dans la Div. 1F, à la suite de la découverte dans cette région de nouvelles agrégations de *S. mentella*. L'exploitation de la Div. 1F pourrait avoir des impacts du côté européen en exerçant une pression de pêche supplémentaire sur une composante du stock de la mer d'Irminger. Par voie de conséquence, cette exploitation pourrait influencer le stock canadien de la mer du Labrador.

Le golfe du Saint-Laurent (Unité 1) et le chenal Laurentien (Unité 2) sont pour l'instant considérés comme deux unités de gestion distinctes. L'Unité 1 est actuellement sous moratoire, à cause de la très faible abondance de la ressource et d'une quasi absence de recrutement, alors que l'Unité 2 continue à soutenir une pêche. Il a été montré que les sébastes des deux unités se regroupent dans la région du détroit de Cabot (Power, 2003), pendant la période de copulation qui a lieu en automne (Ni & Templeman, 1985). En outre, pour les deux espèces, les microsatellites montrent très peu de différences génétiques entre les deux unités, ce qui questionne la validité de la délimitation actuelle (Roques *et al.*, 2001). Cette question est d'ailleurs considérée comme prioritaire par le Conseil pour la conservation des ressources halieutiques (CCRH, 2003).

Les microsatellites montrent également peu de différences génétiques entre les individus *S. mentella* de la mer du Labrador et des Grands Bancs de Terre-Neuve, ce qui implique un lien entre ces régions (Roques *et al.*, 2002). Le Bonnet Flamand n'a pas été étudié à l'aide de microsatellites, mais les données de MDH (Valentin *et al.*, 2006) et océanographiques (Kudlo *et al.*, 1984 et références incluses), ainsi que la morphométrie (Saborido-Rey, 1994), suggèrent que les sébastes de cette région se démarquent de ceux des Grands Bancs.

Plus au sud, les microsatellites montrent des différences entre *S. fasciatus* du plateau de la Nouvelle-Écosse et du golfe du Maine, ce qui légitime la délimitation en deux zones de gestion distinctes. Le stock de sébaste du golfe du Maine n'a cessé de décliner au cours des années 1980. Cependant, la biomasse du stock semble avoir augmenté dans le milieu des années 1990, une tendance qui s'est accentuée depuis 1996. La plupart des individus associés à cette augmentation sont des petits poissons immatures produits localement au début des années 1990 (Mayo, 2000; Northeast Fisheries Science Center, 2001). Tout récemment, une augmentation relativement brutale de la biomasse a été observée pour plusieurs classes d'âge. Cette augmentation est en contradiction avec le modèle de recrutement local, ce qui suggère une origine extérieure au golfe du Maine (Clark & O'Boyle, 2001; Northeast Fisheries Science Center, 2001) et donc un lien avec les unités de gestion adjacentes. Ce lien pourrait être épisodique et dépendre de conditions océanographiques inhabituelles pour la région. En effet, en 1998, année pour laquelle on soupçonne une immigration importante de sébastes dans le golfe du Maine, l'eau de la

pente du plateau continental originaire de la mer du Labrador s'étendait assez loin vers le sud pour entrer dans le golfe du Maine, ce qui ne s'était pas produit depuis les années 1960 (Clark & O'Boyle, 2001).

Par ailleurs, la présence d'une sous-espèce, *S. fasciatus kellyi*, a été répertoriée dans le golfe du Maine sur la base de caractères morphométriques et morphologiques, dont le plus distinctif est la couleur verte-foncée à noire qui contraste avec la couleur rouge habituelle pour *S. fasciatus* (Litvinenko, 1979). La distribution de *S. f. kellyi* tend à se restreindre aux eaux côtières peu profondes (quelques mètres) d'Eastport, dans la baie de Fundy. Les conditions distinctes entre l'embouchure de la baie de Fundy et les eaux adjacentes expliqueraient l'isolement et l'évolution de *S. f. kellyi* (Litvinenko, 1979). Selon Kenchington (1984), la présence d'autres sous-espèces dans diverses zones côtières peu profondes du golfe du Maine n'est pas à exclure. À ce jour, aucun critère génétique n'a été utilisé pour évaluer le statut taxonomique de *S. f. kellyi* ni pour étudier la structure de la population de sébaste du golfe du Maine.

1.11 STRUCTURE ET DYNAMIQUE DE L'HYBRIDATION INTROGRESSIVE ENTRE *S. FASCIATUS* ET *S. MENTELLA* DANS UN CONTEXTE D'ÉVOLUTION

L'étude des populations de sébaste de l'Atlantique du nord-ouest s'intègre également dans une problématique d'évolution, à cause de la présence d'hybridation

introgressive entre *S. fasciatus* et *S. mentella*. L'étude de Roques *et al.* (2001) utilisant les microsatellites a conduit à plusieurs constats sur les implications de l'hybridation introgressive pour la structure des populations dans l'Atlantique nord-ouest et pour l'intégrité des espèces. Premièrement, l'hybridation introgressive n'a pas lieu sur l'ensemble de la zone de sympatrie et est restreinte au golfe du Saint-Laurent et au chenal Laurentien. Deuxièmement, l'introgression (c.-à-d. l'incorporation des gènes d'une espèce dans l'autre espèce) est bidirectionnelle et se traduit par la présence d'un groupe d'individus introgressés dans chacune des espèces. Ces deux groupes ne sont génétiquement pas intermédiaires entre les espèces parentales. Chacun se rapproche de son espèce parentale respective, suggérant l'absence d'hybrides de première génération. Il faut toutefois rappeler que la sélection d'individus, possédant des critères d'identification traditionnels (AFC, EGM et MDH) convergents, a pu conduire à sous-estimer la variabilité génétique. Troisièmement, l'introgression est plus importante chez *S. mentella* et donc asymétrique. Dernièrement, une réduction de la distance génétique est observée entre les deux espèces dans la zone d'hybridation introgressive. Cependant, l'intégrité génétique des espèces parentales est maintenue en dehors de cette zone.

Une récente étude réalisée sur plus de 8 000 individus a décrit la distribution des trois critères usuels de discrimination d'espèce (MDH, AFC, EGM) sur l'ensemble du nord-ouest Atlantique (Valentin *et al.*, 2006). Le génotype au locus de la *MDH-A** a servi à assigner chaque individu à une espèce, tel que décrit plus haut, et les hétérozygotes ont été considérés comme des hybrides. La congruence des caractères méristiques (AFC et EGM)

avec la *MDH-A** a été utilisée pour évaluer le niveau d'introgression de chacune des espèces, dans les différentes unités de gestion. Bien que cette approche soit moins puissante que celle utilisant les microsatellites, elle a confirmé que le processus d'hybridation introgressive est bidirectionnel, asymétrique et restreint au golfe du Saint-Laurent et au chenal Laurentien. Ce dernier point a été révélé par le fait que les hétérozygotes au locus *MDH-A** se retrouvent uniquement dans ces deux régions.

D'après Roques *et al.* (2001), la présence d'un important processus d'hybridation introgressive géographiquement restreint au golfe du Saint-Laurent et au chenal Laurentien, la persistance de deux groupes introgressés en sympatrie et le maintien de l'intégrité des espèces en dehors de la zone d'hybridation introgressive de même que la faible représentation d'hybrides à caractères intermédiaires suggèrent que l'hybridation introgressive entre *S. fasciatus* et *S. mentella* est soumise à une forme de sélection. Cette hypothèse est appuyée par l'étude de déséquilibre de liaison qui révèle que l'association non aléatoire des allèles entre les loci est plus importante chez certains groupes (Roques *et al.*, 2001). Il faut préciser que les microsatellites sont considérés comme des loci neutres, puisqu'ils ne sont pas associés à une fonction particulière. Pour cette raison, ils ne sont pas directement soumis au processus de sélection. Cependant, ils tendront à être sélectionnés passivement s'ils se trouvent à proximité d'un locus sélectionné. Ainsi, dans le cas du sébaste du golfe du Saint-Laurent et du chenal Laurentien, il est possible que les fréquences alléliques observées à divers loci microsatellites reflètent un processus de sélection dépendant de la valeur adaptative différentielle des groupes hybrides et parentaux.

Toujours d'après Roques *et al.* (2001), des conditions océanographiques propres au système golfe du Saint-Laurent–chenal Laurentien pourraient favoriser une forme de sélection exogène (médiée par l'environnement externe à l'individu). À l'opposé, la faible proportion d'hybrides à génotype intermédiaire pourrait signifier la présence d'un mode de sélection endogène (médiée par l'environnement interne de l'individu). Une étude de paternité comparant le génotype au locus de la *MDH-A** a également suggéré la possibilité d'une sélection non aléatoire du partenaire lors de la reproduction (Gagné, 1995). Chez les sébastes de l'Atlantique nord, l'étude des processus de sélection est limitée par l'impossibilité de réaliser des croisements artificiels ou des observations en milieu naturel. Cependant, il est possible de comparer diverses caractéristiques qui composent la valeur adaptative, en l'occurrence l'asymétrie fluctuante et la fécondité, entre les différents groupes hybrides et parentaux.

Dans la présente étude, des considérations techniques et des contraintes de temps n'ont pas permis d'évaluer la fécondité, ni de réaliser une étude de paternité, tel que prévu initialement. Par ailleurs, la caractérisation de l'asymétrie fluctuante a dû être abandonnée, faute de trouver des variables adéquates. Pour toutes ces raisons, le volet du projet visant à tester l'hypothèse de sélection a dû être abandonné. La présente étude se concentre donc sur la caractérisation de la structure des populations. Les objectifs initiaux de cette partie du projet ont été redéfinis en tenant compte des nouvelles hypothèses qui ont émergé pendant la réalisation de l'étude.

1.12 OBJECTIFS DE RECHERCHE

Cette étude vise à décrire la structure des populations de *S. fasciatus* et *S. mentella* de l'Atlantique du nord-ouest, en étudiant les caractéristiques génétiques et morphométriques des grandes agrégations qui soutiennent la pêche. L'étude sert également à documenter le processus d'hybridation introgressive. Dans l'ensemble, elle contribue à fournir de l'information utile dans un contexte de gestion des stocks et d'évolution. Sur le plan méthodologique, l'étude consolide les études antérieures en introduisant des microsatellites additionnels et en utilisant de nouvelles procédures statistiques. Elle offre également une discussion sur la puissance, les limites et la complémentarité des approches génétiques et morphométriques utilisées.

Le même jeu de données est utilisé pour les trois études qui composent la thèse. Il comprend 36 échantillons (19 *S. fasciatus*, 16 *S. mentella* et 1 *S. marinus*), composé chacun d'une trentaine d'individus adultes des deux sexes. Pour chaque individu, le jeu de données contient l'information sur la forme du corps et le génotype à 13 microsatellites. Deux jeux de données supplémentaires sont utilisés dans la première étude (chapitre 2) pour répondre aux objectifs spécifiques à celle-ci. Les différents jeux de données sont décrits en détails dans les différents chapitres.

Le deuxième chapitre traite d'un problème d'artefact rencontré au cours de l'analyse des données morphométriques À première vue, l'artefact découlait de la position du

spécimen au moment de la capture des données. Le premier objectif de l'étude consiste à vérifier que l'artefact est indépendant de facteurs biologiques (taille, espèce) ou du mode de conservation (congélation) des spécimens. Le deuxième objectif est de proposer une méthode pour modéliser l'artefact et l'éliminer mathématiquement des données originales.

Le troisième chapitre se concentre sur les questions relatives aux relations interspécifiques, sur la base des données génétiques et morphométriques. Il traite également de la puissance discriminante et des limites des méthodes génétiques et morphométriques utilisées. Le premier objectif est de déterminer l'implication du nombre et du polymorphisme des loci microsatellites pour la discrimination des espèces. Le second objectif vise à documenter l'hybridation introgressive entre *S. fasciatus* et *S. mentella* et à évaluer la possibilité d'une hybridation impliquant *S. marinus*. Un troisième objectif consiste à évaluer le pouvoir discriminant et la convergence des approches génétiques et morphométriques.

Le quatrième chapitre se concentre sur la structure et la connectivité des populations de *S. fasciatus* et *S. mentella*, telles que définies par les données génétiques et morphométriques. L'analyse d'un échantillon provenant du fjord de Bonne Bay donne l'opportunité d'évaluer empiriquement les conséquences évolutives de l'isolation d'une population. Cette estimation fournit un point de référence auquel comparer les valeurs de différenciations génétique et morphométrique observées entre les autres populations. L'étude discute de l'implication des facteurs environnementaux et de l'hybridation

introgressive dans la détermination de la structure des populations. Elle offre également une discussion sur la pertinence biologique des unités de gestion.

CHAPITRE 2

ARCHING EFFECT AFFECTING FISH BODY SHAPE IN GEOMETRIC MORPHOMETRIC STUDIES

2.1 ABSTRACT

Several geometric morphometric studies on fish have revealed the presence of shape variability in the data that corresponds to an upward/downward arching of the body. Such arching was observed during a study on redfish (*Sebastes* sp.) population structure in the northwest Atlantic. The arching was a dominant source of variation, generating more variability than the interspecific differences. The present study investigates the potential causes of this arching. The results suggest that it is not related to biological factors (size, species, and condition) or to the preservation technique (freezing). It is proposed that the arching is an artefact resulting from slight posture variations between fish during landmark capture. The consequences of the arching artefact on data analysis are discussed. An approach coupling a PCA-based model of the arching with Burnaby's orthogonal projection is proposed for removing the artefact from the data.

Keywords: shape; arching artefact; geometric morphometrics; Burnaby's projection; redfish.

2.2 INTRODUCTION

Traditionally, biological morphometric studies were based on measurements of anatomical traits of which mean values could be compared between groups of specimens. In the nineties, the development of shape theory, the recognition of landmarks as manageable data, and the development of many software and digitization programs led to a new field in morphometrics called "geometric morphometrics" (Rohlf & Marcus, 1993; Marcus & Corti, 1996; Adams *et al.*, 2004). Landmarks capture the locations of anatomical characteristics, and their relative positions indicate shape differences between specimens. In geometric morphometrics, landmarks are coded as 2D or 3D coordinates. These coordinates are superimposed using Generalized Procrustes Analysis (making them invariant to scale, location, and orientation) and transformed into shape variables that can be analysed with standard multivariate statistics (Adams *et al.*, 2004, and references therein). The relative spatial arrangement of landmarks is conserved throughout the analysis, which allows an easily interpretable visualization of morphological variability. New developments are expected, but it is now widely accepted that geometric morphometrics has reached a mature phase. There is a strong consensus toward a standard protocol based on Procrustes methods that is recommended for most applications (Adams *et al.*, 2004).

Geometric morphometrics can be performed on living, dead, or even fossil material. Along with genetics, which took advantage of the development of molecular techniques, geometric morphometrics has become a powerful tool for biologists and palaeontologists

(Elewa, 2004). This approach is now routinely applied to a large variety of taxa in studies dealing with various issues in evolution and systematics, such as species discrimination (e.g., Cardini & O'Higgins, 2004), natural selection (e.g., Claude *et al.*, 2003), ontogeny (e.g., Berge & Penin, 2004), asymmetry (e.g., Klingenberg *et al.*, 2002), and many more. The popularity of geometric morphometrics is reflected by the increasing number of publications over the last decade (Adams *et al.*, 2004).

Finding landmarks that are homologous between specimens is an important prerequisite to making meaningful shape comparisons. Another important factor is to ensure the accuracy of landmark capture. The recent development of "sliding semilandmarks" has allowed the compensation for the absence of precise anatomical features along curves (Bookstein, 1997), but the issue of accuracy in capturing landmarks remains problematic. Indeed, although geometric morphometrics is not based on distance measurement, it is still subject to digitization error. Causes and consequences of measurement errors are now widely recognized (Arnqvist & Mårtensson, 1998, and references therein), but most empirical geometric morphometric studies do not address this issue, except perhaps for studies of fluctuating asymmetry where measurement errors are routinely monitored (Hallgrímsson *et al.*, 2003; Palmer & Strobeck, 2003). Discussing all possible sources of measurement error would be beyond the scope of this paper, and the focus will only be on those relevant to the present study (for a complete discussion see Arnqvist & Mårtensson, 1998, and references therein).

When 3D specimens are reduced to a 2D representation, digitization errors will occur if specimens do not lie in exactly the same horizontal plane relative to the axis of the dimension being reduced (as an example, think of how the shape of an object changes when you look at it from different angles). The use of any optic device to generate a 2D representation of a 3D specimen may lead to additional digitization errors if the photographic settings are not standardized between specimens. Indeed, a lens causes optical distortion (known as parallax) because it generates an image through a central projection of the original 3D object. The amount of distortion varies according to the position of the camera relative to the specimen being photographed. The situation is worse for close-up shots with structures of interest that are not on the same plane. The importance of lens quality and photographic setups for minimizing the bias of morphometric data is now widely recognized (Arnqvist & Mårtensson, 1998; Mullin & Taylor, 2002; Frieß, 2003).

An individual landmark configuration will also depend strongly on the specimen's posture during digitizing (Arnqvist & Mårtensson, 1998). Depending on the kind of specimens under study, achieving a standard or neutral posture for each individual is not straightforward. Fish provide a good example of this issue. Usually, there is no difficulty in defining precise landmarks on the specimens. However, the body of a fish is not a rigid structure, and specimen shape will be influenced by its posture during landmark capture. Preparation of the specimens before digitizing is therefore a critical step. It is influenced by the nature of the specimen under study and by its mode of preservation. For example, in the case of fish, freezing as well as formaldehyde are used as preservatives. Everyone who has

manipulated a fish preserved in formaldehyde can appreciate to what extent shape may be affected by preservation (e.g., flexion, shrinkage).

Slight posture differences between specimens during digitizing may be considered as acceptable when measurement errors translate into randomly distributed variability that is small relative to the real between-individual variance in shape. Indeed, most statistical models are based on the ratio of explainable to residual variance. When measurement error is low (in comparison with explainable variance) and randomly distributed, the resulting loss of statistical power will not be problematic. However, when variability is unevenly distributed, a bias is to be expected (Arnqvist & Mårtensson, 1998). Several studies on fish have underscored the presence of shape variability in the data corresponding to an upward/downward arching of the body. Usually, this arching was considered as an artefact caused by specimen preservation (Carpenter, 1996; Cavalcanti *et al.*, 1999). To our knowledge, no study has tested this hypothesis.

Few studies have considered the possibility of removing bias from morphometric data, but there is an increasing effort driven by palaeontologists working with deformed fossils (Motani, 1997; Angielczyk *et al.*, 2004; Dunlavey *et al.*, 2004). Different methods have also been explored to remove variability from the data caused by the arbitrary positioning of articulated structures (Adams, 1999) and by head orientation in side-view images of human heads (Gharaibeh, 2005). For the above-mentioned arching effect, there is a program that fits a quadratic curve through a designated set of landmarks and then

"unbends" the entire configuration so that the estimated quadratic curve becomes a horizontal straight line (available in the tpsUTIL software, Rohlf, 2003a). Unfortunately, such bias is often detected after data analysis, and data reacquisition to define this set of landmarks is not always possible.

An upward/downward arching has been detected in the morphometric data set of an ongoing multidisciplinary study on redfish population structure in the northwest Atlantic. The northwest Atlantic redfish consists of a complex of three species currently identified as *Sebastes fasciatus* and *S. mentella,* which undergo introgressive hybridization and support a commercial fishery, and *S. marinus*, which occurs at much lower abundance. There has been no substantial recruitment in the last 20 years, resulting in the collapse of the fishery without a significant sign of recovery. In this context, it became important to understand the structure and the inter-relationships of the redfish fishing stocks for developing sound management strategies. The 1996-1998 Mutidisciplinary Research Program on Redfish (Gascon, 2003) and related studies (Roques *et al.*, 2001 and 2002; Valentin *et al.*, 2002) gave new insights regarding the redfish population structure in the northwest Atlantic. However, additional investigations were needed to improve the knowledge on this complex question. This led to the ongoing multidisciplinary study coupling genetics, otolith elemental fingerprints, and geometric morphometrics. The upward/downward arching appeared during a preliminary analysis of the morphometric data set. Since the magnitude of this effect was important compared to the overall shape variability, the upward/downward arching was investigated extensively.

The first objective of the present study was to investigate whether biological factors (size, species) or specimen preservation (freezing) was responsible for the upward/downward arching observed in the morphometric data set. The second objective was to propose an alternative to Rohlf's unbending method for removing this bias by using a model of the associated body deformation.

2.3 MATERIAL AND METHODS

2.3.1 Samples

Three sample sets were used in the present study. The first one consisted of 27 fish collected in May 2001 in the St. Lawrence estuary. Their size varied between 20 cm and 34 cm. They served to assess the effect of freezing/thawing on body shape. This part of the study was performed as a preliminary test for the multidisciplinary research on redfish population structure in the northwest Atlantic. At first, the project was designed to work mostly on fresh specimens and also on some frozen samples from remote areas. For the multidisciplinary study, this strategy was changed so that only frozen samples were used to minimize the shape variation associated with different specimen handlings.

Specimens of the second sample set were those from the large-scale population study on which the upward/downward arching was first observed. This sample comprised

1121 specimens collected across NAFO fishing areas in 2001 and 2002. They included male and female adult redfish ranging in size from 22 cm to 51 cm, caught either in summer or in fall. Fish were identified as *S. fasciatus* (N = 596), *S. mentella* (N = 495), or *S. marinus* (N = 30) according to their genetic characteristics at 13 microsatellite loci (see Chapter 3). For convenience, this sample is referred to as the "population structure data set."

The third sample consisted of a single redfish randomly chosen from among the extra specimens caught during the 2002 sampling campaign. It was used to generate the deformation model that allowed the removal of the arching effect from the population structure data set.

All specimens were collected with a bottom-trawl net (except for 31 redfish caught using handlines during ice fishing). For the fresh–thawed comparison study, all freshly caught specimens were decerebrated before a first set of morphometric data was collected (see below). Then the specimens were frozen individually and brought to the laboratory for further data collection. Specimens from the population structure data set and the single one for the model were laid flat in cardboard boxes and frozen immediately on board pending data collection, which was performed in the laboratory.

2.3.2 Landmark capture

Ten landmarks (L_1 to L_{10}) defining the body outline were selected (Figure 2.1). Prior to data collection on fresh or thawed specimens, the inflated gasbladder and air pockets in the eyes and mouth, caused by the rapid ascent of the trawl, were deflated and stomach was put back in place when evaginated. Then, the mouth was kept closed with a rubber band and fin membranes were cut with a scalpel to gain access to hidden landmarks (L_4 and L_7). Finally, the fish was laid on its right side, in its most natural posture.

For the fresh–thawed comparison study, each of the 27 freshly caught fish was photographed on board with a digital camera (Olympus, model No. C-3000 ZOOM) and then frozen in an individual plastic bag. A second picture was taken after thawing, in the laboratory. This led to a data set of $N = 54$. It was often difficult to keep the fish in a natural posture without holding it by hand (especially in the head area), suggesting that taking pictures might not be appropriate for capturing data for this species. Some landmarks were also difficult to recognize on the pictures (for example, L_1 and L_2 were respectively replaced by L_{1a} and L_{2a} for the fresh–thawed comparison study). For further studies, this imposed the time-consuming precaution of marking each landmark with a black-headed pin before taking picture.

For the population structure data set ($N = 1121$), each fish was laid on a styrofoam board covered with a piece of waxed paper. The position of each landmark was determined

by punching a hole in the paper with a needle. A support was used to maintain the needle perpendicular to the board. Each waxed paper was then digitized. This approach was more appropriate than taking pictures considering the large number of specimens and the difficulty of laying the fish in a natural posture.

Finally, for the deformation model study, a black-headed pin was inserted in the fish at each landmark. Then, the left side of the same specimen was photographed 12 times in different arching postures with a digital camera. To optimize the accuracy of the model, arching was not exaggerated, but limited to the range covered by fish from the population structure data set during their landmark capture. This strategy allowed to generate a data set (N = 12) in which variation was only related to body posture.

Landmark digitizing was done using the tpsDIG software (Rohlf, 2003b): x,y coordinates were determined for each landmark of a given specimen, providing a total of 20 morphometric variables. A ruler present on each picture served to determine the scale factor.

2.3.3 Geometric morphometrics and multivariate statistics

Each three sets of coordinates (i.e., fresh–thawed comparison, N = 54; population structure data set, N = 1121; deformation model, N = 12) were submitted

separately to a Generalized Procrustes Analysis (GPA) available in the APS (Penin, 2001) and tps RELATIVE WARPS (Rohlf, 2003c) software. This procedure translated, rotated, and scaled (to unit centroid size) the original configurations in order to achieve the best superimposition of all shapes. For each three data sets, aligned coordinates (considered as shape data) were introduced in three separate analyses of tps RELATIVE WARPS (Rohlf, 2003c), an equivalent of principal component analysis (PCA) for shape data. PCA eigenvectors were saved. For each PCA, shape changes associated with each principal component (PC_i) and shape differences between specimens were visualized either with vector displacement (provided by the APS and tps RELATIVE WARPS software) or grid deformation (provided by the tps RELATIVE WARPS software).

As mentioned above, the variability for the deformation model was only related to body posture since the same specimen was digitized in different bending positions. This translated into a first eigenvector summarizing most (97%) of the total variance and describing the upward/downward arching. The angle between the first eigenvector of the deformation model and the eigenvectors of both the fresh–thawed comparison and the population structure studies were computed. This allowed us to assess whether bending was a dominant or an incidental source of variation and whether its effect on shape was linear in shape space. A small angle would indicate a strong effect of the deformation on the component. So, if small angles were mostly found for components summarizing an important amount of variance (i.e., PC_1 and the few following components), this would indicate that bending is dominant and must be removed. Moreover, if only one component

was affected, this would be a sign that the effect is mostly linear in shape space. Actually, results showed that the upward/downward arching was essentially affecting only the first component of both the fresh–thawed comparison and the population structure studies. This property was instrumental in choosing the statistical procedure, which included univariate statistics on the PC_1 scores and discriminant function analyses (DFA) on the PC scores.

Usually, partial warp scores are used as input data for DFA. Using PC scores (also known as relative warp scores) gives identical results in term of statistics, since these variables are only separated by rigid rotation, but the interpretation of the shape changes is limited. Principal component analysis is a method of dimension reduction. PCA axes are necessary to capture the space, but they will not necessarily be parallel to interesting trends. Therefore, interpreting PCA axes individually is not recommended and PC scores are usually not analysed through a DFA. In the present study, the fact that the interesting effect (upward/downward arching) was linear and concentrated on PC_1 allowed the following approach. PC scores were used as shape data in DFA, testing differences between groups for a particular factor (e.g., preservation method). A PC_1 with a very low weight on the discriminant function would be an indication that bending was independent of the effect being tested. The interpretation of the weight of the other PC is less interesting *per se*, but it helps to identify the PCA components describing the space where the factor has an important effect. The property of the PC_1 to summarize the bending also offered the opportunity to use it as a dependent variable on which several effects (size, species, sex, etc.) were tested through univariate statistics.

For the fresh–thawed comparison study, the effect of preservation on PC_1 was tested through a single factor ANOVA. In addition, a discriminant analysis (DFA) was performed on the principal component scores. These scores were thus combined in a linear function that best discriminated individuals of the two groups (i.e., fresh vs thawed) on the basis of their body shape. A jackknife procedure was used to assess classification efficiency obtained by the discriminant function. A comparison of means was carried out between the two groups through MANOVA. Shape variations associated with the discriminant function were visualized with tpsREGR (Rohlf, 2000) using the vector of DFA scores as the independent variable. DFA versus PC_1 scores were plotted.

For the population structure data set, an ANCOVA in which PC_1 was regressed on the centroid size within each species was performed to test whether the upward/downward body arching summarized by the first component was related to allometry or species. A DFA (including a jackknife) procedure was performed on the principal component scores to further investigate whether PC_1 was independent of species differences. It also helped to identify the PCA components describing the space where shape differences were significant between species.

Burnaby's method, as provided by the NTSYSpc version 2.10y software (Rohlf, 2002), was used to remove the arching artefact from the population structure data set. Originally, this method was designed for removing general size from a set of linear distance measurements to generate a data set in which shape variation was independent of size

(Burnaby, 1966). For this purpose, the size vector was projected into a complementary subspace. This subspace is defined through matrix algebra using the following formula:

$$L = I_q - f_1 (f_1' f_1)^{-1} f_1'$$

For N specimens and q variables, $\mathbf{f_1}$ is a $q \times 1$ column vector representing size or any extraneous variable one wants to remove from the data set. $\mathbf{I}$ is a $q \times q$ identity matrix, and $\mathbf{L}$ is a $q \times q$ matrix of rank $q - 1$ (Rohlf & Bookstein, 1987). Multiplying the data matrix by $\mathbf{L}$ generates a set of adjusted specimens spanning only the hyperplane where variation is orthogonal to the original vector $\mathbf{f_1}$. In the present study, the aligned coordinates (representing shape variables) have been projected orthogonally to the first eigenvector of the deformation model (representing $\mathbf{f_1}$).

The projected coordinates were submitted to a GPA followed by a PCA. Shape changes associated with the new principal components (PC_{Bi}, where "B" stands for "Burnaby," to avoid confusion with PC_i, the principal components issued from the PCA before correction) were been visualized. Then, the same statistical procedure as the one performed on the population structure data set before correction was followed for analysing scores of these new components. It involved an ANCOVA in which PC_{B1} was regressed on the centroid size within each species to test the effect of allometry and species on the new first component (i.e., the axis of maximum variability). It also included a DFA (with a jackknife procedure) on the PC_{Bi} between the three species, generating two discriminant functions or axes. This procedure helped to single out PCA components describing the space where species discrimination was dominant. DFA scores of the two discriminant

functions were plotted. Finally, tpsREGR (Rohlf, 2000) was used, with the matrix of DFA scores of the two functions as the independent variable, to explore shape variations associated with the discriminant functions.

2.4 RESULTS

As expected for the deformation model, PC_1 summarized most (97%) of the total variance. Figure 2.2 shows the upward/downward body arching associated with this component. The angle between the first eigenvector of the model and the first eigenvectors of both the fresh–thawed comparison and the population structure studies were respectively 23.9 and 14.9 degrees. This suggested that the model successfully described the arching deformation associated with the PC_1 for both data sets. Values for the angle between the first eigenvector of the model and the eigenvectors of the remaining components (i.e., PC_2 to PC_{16}) of both data sets were homogeneous, with a mean of 92.3 degrees (SD = 5.7) for the fresh–thawed comparison data set and 89.5 degrees (SD = 3.9) for the population structure data set. These values close to 90 degrees meant independence between PC_1 of the model and these components. As mentioned above, these results indicated that the effect of the upward/downward arching was mostly linear in shape space and that this arching was a dominant source of variation.

For the fresh–thawed comparison study, the first three principal components explained 44%, 16%, and 7% of total variance, respectively (Table 2.1). The arching deformation was observed on PC_1 (Figure 2.3) and was independent of preservation (ANOVA: $P = 0.58$; $F = 0.30$; df = 1, 52; $R^2 = 0.0058$). PC_2 was mostly related to sex differences (not shown), as confirmed by a single-factor ANOVA ($P < 0.0001$; $F = 68.0$; df = 1, 50; $R^2 = 0.58$). Shape differences between thawed and fresh specimens were concentrated on PC_3 and PC_6 (not shown). This suggested that freezing specimens did not generate body arching, and this was confirmed by the DFA performed on the PCA scores between the fresh and thawed groups. Indeed, the highest weights on the discriminant function (Table 2.1) were found for PC_3 ($F = 36.1$; df = 1, 37) and PC_6 ($F = 21.0$; df = 1, 37), while PC_1 was negligible ($F = 0.91$; df = 1, 37). The independence between the effects of arching (summarized by PC_1) and preservation (summarized by the DFA axis) is illustrated in Figure 2.3. According to the MANOVA, shape differences between the fresh and thawed groups were significant ($P < 0.0001$; $F = 7.4$; df = 16, 37) and mostly localized in the head area (L_1, L_2, and L_{10} ; Figure 2.3). The discriminant function could classify fresh and thawed specimens in their respective groups with a 98% success rate. However, the jackknife value was lower (85%), suggesting that the model had too many predictors.

For the population structure data set (prior to the Burnaby projection), 47%, 19%, and 8% of the total variance were explained by the first three principal components, respectively (Table 2.1). The first component (PC_1) was associated with body arching (Figure 2.4). The ANCOVA showed significant interaction between centroid size and

species on PC_1 ($P = 0.0002$; $F = 8.7$; df = 2, 1115), but with a very bad fit ($R^2 = 0.035$). Regressions of centroid size on PC_1 within species were significant for *S. marinus* ($P = 0.0005$; $F = 15.7$; df = 1, 28; $R^2 = 0.36$) and *S. fasciatus* ($P = 0.0008$; $F = 11.4$; df = 1, 594; $R^2 = 0.019$), but not for *S. mentella* ($P = 0.46$; $F = 0.5$; df = 1, 493; $R^2 = 0.001$). In each case, the regression model fit the data poorly, suggesting no strong correlation between PC_1 and centroid size. No other biological variable, such as sex or maturity, nor geographic factor were explanatory (not shown). PC_1 had a low weight ($F = 17.8$; df = 2, 1103) in the DFA between species, further indicating that body arching was not related to species differences (Table 2.1). PC_2 ($F = 1758.8$; df = 2, 1103) and PC_3 ($F = 345.9$; df = 2, 1103) had the highest weights, suggesting that PC_2 (Figure 2.4) and, to a lesser extent, PC_3 summarized interspecific differences, especially those between *S. fasciatus* and *S. mentella*. Altogether, these results suggested that body arching was a dominant source of variation (more important than interspecific shape differences) with no biological interest, and, therefore, that it must be removed from the data.

For the PCA performed on the population structure data set coordinates after their Burnaby projection on the model first eigenvector, percents of total variance explained by the first three principal components were respectively 34%, 15%, and 12% (Table 2.1; Figure 2.5). The full ANCOVA model on PC_{B1} with species as factor and centroid size as covariate had an R^2 of 0.67. The interaction between centroid size and species was significant ($P = 0.008$; $F = 4.8$; df = 2, 1115), but only because of the 30 *S. marinus* specimens. After removing these specimens, centroid size ($P < 0.0001$; $F = 283.9$; df = 1,

1088) and species ($P < 0.0001$; $F = 2275.9$; df = 1, 1088) were both significant for the model without interaction ($R^2 = 0.68$). This indicated that shape changes summarized on PC_{B1} were mostly species specific and, to a lesser extent, allometric. DFA indicated that PC_{B1} summarized most of the interspecific differences, especially those between *S. fasciatus* and *S. mentella* (Figure 2.5), since PC_{1B} had the highest weight in the analysis ($F = 1759.4$; df = 2, 1104 ; Table 2.1). A discriminant function based only on PC_{B1} could correctly classify 89% of the specimens for these two species. Shape differences were mostly related to landmarks L_3 and L_8, suggesting a more fusiform shape for *S. mentella* compared to *S. fasciatus* (Figure 2.6). *S. marinus* showed expansion at landmark L_4, suggesting a higher body depth in the posterior area than the other two species. Compression in the head area at landmarks L_2, L_3, and L_{10} suggested a relatively smaller head for *S. marinus* (Figure 2.6).

2.5 DISCUSSION

The model's high level of accuracy in describing the observed arching was the biggest element of surprise in this study. If we consider the way it was generated (i.e., by modelling a fish covering the range of postures achieved by specimens during landmark capture), it strongly suggests that the observed upward/downward arching was related to slight posture variations between fish during landmark capture. This hypothesis is reinforced by all the inconclusive attempts to explain these posture variations by biological

factors. Indeed, neither allometry, nor species, nor sex were explanatory. For *S. marinus*, however, the correlation between PC_1 and centroid size was slightly higher than for the two other species. The 30 *S. marinus* specimens were the largest individuals in our sample (mean = 44.0 cm, SD = 3.7). This suggests that for large specimens (over 35 cm), size might slightly influence the fish's posture during landmark capturing, resulting in bigger fish exhibiting a relatively more U-shaped body line. Our data set does not allow further investigation, but this effect could be related to the fact that bigger specimens tend to be relatively less flat (i.e., more three-dimensional) than smaller ones, which could influence their posture once laid flat. It has to be remembered that landmark coordinates were collected by punching holes in a waxed paper, so this change of posture is not related to distortion generated by the use of an optic device.

Fish condition could be another biological explanation of the observed deformation. Fulton's index is a common measure of fish condition. It is based on a weight/length ratio, measuring the plumpness for a given length (Lagler, 1956). Two separate tests made on two subsets of the data (22 specimens from the Saguenay River, Quebec, Canada, and 22 specimens from West Greenland) for which individual weights were available showed no indications that body arching could be related to condition (not shown). Besides, condition is known to vary between seasons, although the variation is small for northwest Atlantic redfish (Jean-Denis Dutil, Department of Fisheries and Oceans, Mont-Joli, Québec, Canada, pers. comm.), but no seasonal differences in body shape were observed between

summer and fall samples for a given species in a given area (see Chapter 4). All this evidence suggests that fish condition was not responsible for the observed deformation.

Results of the fresh–thawed comparison study further indicated that the deformation was mostly related to random posture variations between fish during landmark capture. Indeed, attempts to relate the observed deformation to specimen preservation were unsuccessful. Namely, the first principal component summarizing the deformation had a very low weight on the discriminant function between the fresh and thawed specimens. Several studies dealing with fish morphology have described a similar body arching and proposed preservation as the responsible effect (Carpenter, 1996; Cavalcanti *et al.*, 1999). The present study tends to invalidate this hypothesis. However, fresh and thawed specimens did exhibit slight but significant shape differences, especially in the head area. Some level of optic distortion due to the use of a camera cannot be ruled out, but a biological explanation is more likely. Indeed, visual observations showed that fresh fish tended to be more inflated in the head and gill area. Contraction of the muscle controlling pectoral fins was also observed. So, the shape differences observed between fresh and thawed specimens would rather be caused by muscle contraction accompanying death than by a direct effect of preservation through freezing. According to the present study, combining fresh and thawed redfish specimens in the same analysis would introduce a bias to the data. This highlights the importance of standardization regarding the handling and preservation of specimens in morphometric studies.

Altogether, the results suggested that the observed upward/downward arching deformation was independent of biological factors or preservation technique, and was rather related to slight posture variations between fish during landmark capture. A hypothesis to explain these slight variations may be death by suffocation. Suffocation has been hypothesized as a factor causing arching deformation (as mentioned in the tpsUTIL help file). According to this hypothesis, our results would suggest that death by suffocation randomly affects body shape of freshly caught fish, and that this particular random effect is independent of preservation (since it remains after freezing and thawing).

In the present study, slight random posture variations between fish during landmarks capture (whatever their cause) generated higher variability than that accounted for by species differences or any other biological factor. Indeed, the arching deformation was summarized by the PC_1 component, accounting for 47% of the total variance, while species differences were relegated to PC_2 (19% of the total variance). Such deformation represents an important bias when exploring shape changes embedded in data. Moreover, this bias may influence the significance of statistical tests (Arnqvist & Mårtensson, 1998). Under these considerations, correcting data was appropriate for the population structure data set of the present study. Orthogonal projection of the aligned coordinates on the first eigenvector of the model was very effective for removing the arching deformation. This was not surprising since the angle between the PC_1 eigenvector of the deformation model and the original first eigenvector of the population structure data set was small (14.9 degrees). The corrected data set gave biologically consistent results, with interspecific

differences brought back to the first component. The corrected redfish data set will serve for the ongoing extensive study on redfish population structure in the northwest Atlantic (see Chapter 4).

This correcting approach coupling a PCA-based model of the observed deformation with Burnaby's projection is not always warranted (Angielczyk *et al.*, 2004) and should be further investigated. However, it has given promising results in various morphometric studies that had unwanted additional variability caused by arbitrary positioning of articulated structures (Adams, 1999) and by head orientation in side-view images of human heads (Gharaibeh, 2005).

2.6 ACKNOWLEDGMENTS

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Table 2.1 Summary of the statistics for the Principal Component Analyses (percent of total variance explained by the components) and the Discriminant Function Analyses (*F*-to-remove for the components) performed on the fresh–thawed comparison data set and the population structure data set, before and after the Burnaby projection. DFA[a] between the fresh and thawed groups (df = 1, 37); DFA[b] between the 3 species (df = 2, 1103); DFA[c] between the 3 species (df = 2, 1104)

	fresh–thawed comparison data set		population structure data set before Burnaby projection		population structure data set after Burnaby projection	
Rank of Principal Component	PCA % of variance explained	DFA[a] F-to-remove	PCA % of variance explained	DFA[b] F-to-remove	PCA % of variance explained	DFA[c] F-to-remove
1	43.5	0.9	46.6	17.8	33.6	1759.4
2	15.7	0.2	18.9	1758.8	15.3	268.0
3	7.3	36.1	7.9	345.9	12.0	179.4
4	7.1	0.3	6.0	112.2	9.5	101.3
5	5.4	3.5	4.4	91.0	7.1	28.6
6	4.9	21.0	3.8	26.4	4.7	13.6
7	3.9	6.3	2.6	12.8	3.8	26.7
8	3.7	8.7	2.1	23.7	3.2	0.6
9	2.3	0.3	1.7	8.1	2.4	72.2
10	2.1	2.6	1.3	62.9	2.0	30.6
11	1.2	9.9	1.1	31.5	1.8	25.0
12	1.0	2.5	1.0	24.0	1.6	0.1
13	0.8	0.0	0.9	0.3	1.4	50.2
14	0.5	7.1	0.8	49.9	1.0	16.1
15	0.5	4.8	0.6	15.7	0.8	19.5
16	0.3	14.9	0.4	19.5	0.0	—

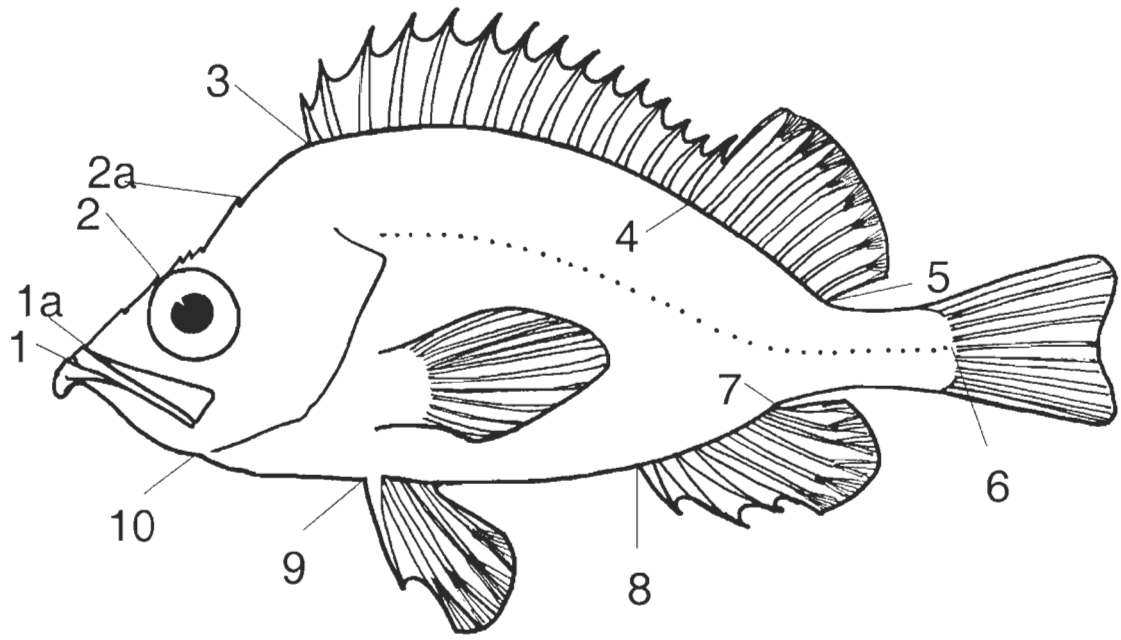

Figure 2.1 Position of the 10 landmarks used to define body shape. L_{1a} and L_{2a} replaced L_1 and L_2 in the comparison study between freshly caught and thawed specimens. (1) bottom of the teeth on the lower jaw; (1a) top of the upper lip; (2) preocular spine; (2a) occipital or parietal spine; (3) anterior insertion of the dorsal fin; (4) posterior base of the last hard ray on the dorsal fin; (5) posterior insertion of the dorsal fin; (6) posterior extremity of the lateral line; (7) posterior insertion of the anal fin; (8) anterior insertion of the anal fin; (9) anterior insertion of the pelvic fin; (10) posterior extremity of the lower jaw (modified from Valentin *et al.*, 2002).

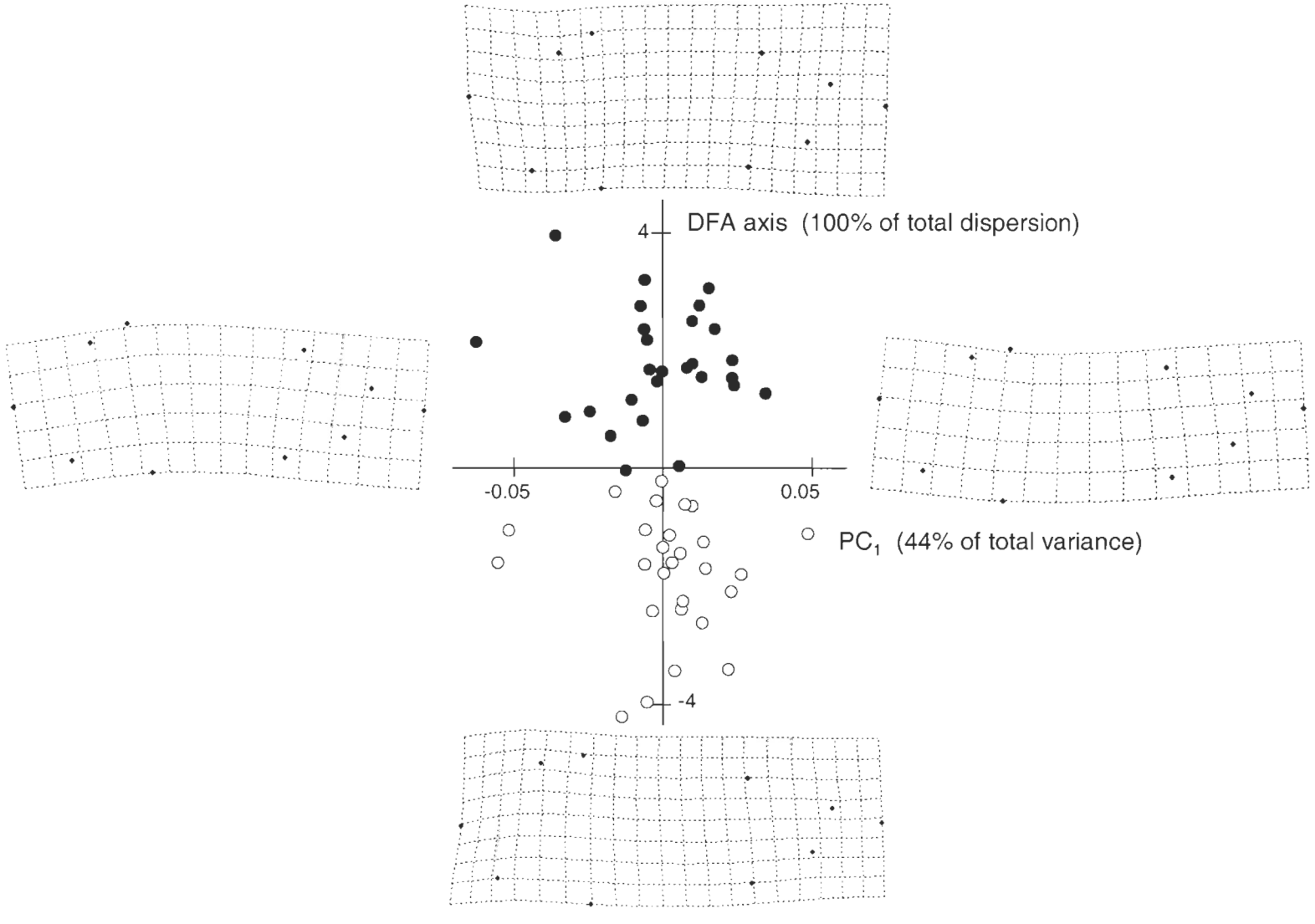

Figure 2.3 PC_1 scores (with % of total variance) versus DFA scores (with % of total dispersion) for the fresh–thawed comparison study. Deformation grids show shape changes associated with PC_1 (i.e., the upward/downward arching) and shape changes (magnified 3 times) between freshly caught (○) and thawed (●) specimens along the discriminant axis.

CHAPITRE 3

COMBINING MICROSATELLITES AND GEOMETRIC MORPHOMETRICS FOR REDFISH SPECIES DISCRIMINATION IN THE NORTHWEST ATLANTIC : METHODOLOGICAL CONSIDERATIONS AND NEW INSIGHTS ON INTROGRESSIVE HYBRIDIZATION

3.1 ABSTRACT

An approach combining genetics and geometric morphometrics has been used to compare the three redfish species, *Sebastes mentella*, *S. fasciatus*, and *S. marinus*, in the northwest Atlantic. The genotype at 13 microsatellite loci, and body shape—defined by 10 anatomical landmarks—were determined on 1121 specimens representing 36 aggregations of redfish across the northwest Atlantic. An overall good concordance was observed between the two data sets. Species discrimination was highly significant for both the genetic and the morphometric approaches. The use of five additional microsatellite loci compared to former studies has improved the discrimination between *S. mentella* and *S. fasciatus*. The index of differentiation F_{ST} by locus was negatively correlated with the locus heterozygosity, indicating that the F_{ST} estimates were constrained by the locus polymorphism. Loci SEB30 and SEB31 outperformed the other loci for species discrimination, according to the G'_{ST} index of differentiation (standardized for the polymorphism). This performance is discussed in relation with the motif at the microsatellite locus and with size homoplasy. Using a Bayesian model-based clustering method on microsatellite data has confirmed previous findings on bidirectional asymmetrical introgressive hybridization between *S. mentella* and *S. fasciatus*. It also suggested that *S. marinus* may be involved in the process. Introgressive hybridization was not associated with species morphological convergence, which contrasted with former findings.

Keywords: microsatellites, geometric morphometrics, body shape, discriminant power, Bayesian statistics, introgressive hybridization, redfish, *Sebastes*, northwest Atlantic

3.2 INTRODUCTION

Microsatellite markers are considered as a major breakthrough for population genetics and species conservation (Jarne & Lagoda, 1996; Beaumont & Bruford, 1999; Sunnucks, 2000). This is reflected by the increasing number of studies using microsatellites over the last decade. These studies have been performed on a long list of species and cover various topics—taxonomic status, hybridization, introgression, population structure, population connectivity, and many more. The popularity of microsatellites is related to several properties, such as a PCR-based assay, a large number of highly polymorphic loci, codominance, and Mendelian transmission. As their use spread, several statistical approaches have been developed to specifically analyze microsatellite data. Basically, differentiation indices and distances based on allelic frequencies have been replaced by indices and distances based on allelic size differences (i.e, accounting for the mutational process). Moreover, the availability of microsatellite data was instrumental in the development of simulation techniques based on maximum likelihood, Bayesian statistics and Markov chain Monte Carlo, which improved the inference made from genetic data (Luikart & England, 1999; Sunnucks, 2000).

In parallel, several studies have underlined various issues associated with analysing and interpreting microsatellite data. For example, it is now recognized that amplification failure and stuttering may lead to genotyping errors (Bonin *et al.*, 2004; Hoffman & Amos, 2005). The properties of microsatellite regarding polymorphism and mutational process

have also raised questions about the validity of sampling design, the accuracy of statistical estimates, and the power of statistical tests (e.g., Ruzzante, 1998; Hedrick, 1999; Balloux & Lugon-Moulin, 2002; Kalinowski, 2005). Polymorphism is an interesting property of microsatellites, because it allows detecting small genetic differences. However, it is expected for less polymorphic loci to yield higher F_{ST} values, because F_{ST} estimates are constrained by the level of heterozygosity. In fact, it has been shown that F_{ST} cannot exceed the amount of homozygosity expressed as 1-H_S, with H_S being the mean within-population heterozygosity (Hedrick, 1999). On the other hand, the increase in the value of the F_{ST} for less polymorphic loci is made at the cost of increased variance, which may reduce the discriminating power for these loci (Kalinowski, 2002). When genetic differentiation is low, locus polymorphism may become limiting in detecting genetic structure even though the estimate has lower variance than less polymorphic loci.

The concern about the sustainability of marine resources has led to an increasing effort in studying population structure in the sea and consequently encouraged the use of microsatellites on marine species, and especially on commercially exploited fish. At the species level, the use of microsatellites often revealed the presence of cryptic and sibling marine species that remained undetected by non-genetic approaches (Knowlton, 2000). Another major asset of microsatellites is their ability to detect and study hybridization. Hybridization appeared to be more frequent than anticipated in marine fish species, and the number of observed hybridizing species increased with the study effort stimulated by commercial concerns (Gardner, 1997).

The structure of Northwest Atlantic redfish stocks has been extensively studied after their major collapse in the mid 1990s. A genetic study, based on eight dinucleotide microsatellites (Roques *et al.*, 1999a; Roques *et al.*, 2001), has confirmed previous knowledge regarding the specific composition of the stocks (Rubec *et al.*, 1991; Gascon, 2003, and reference therein). Three species are recognised in the northwest Atlantic: *Sebastes mentella* (Travin, 1951), *S. fasciatus* (Storer, 1854) and *S. marinus* (Ascanius, 1772). *Sebastes marinus* is considered as of little commercial importance in this area (DFO, 2001*a*). In the northwest Atlantic, *S. marinus* is mostly distributed on the Flemish Cap, and represents less than 1% of the capture in the Gulf of St. Lawrence (Ni & McKone, 1983; Rubec *et al.*, 1991; Gascon, 2003). For the fishery management, *S. fasciatus* (Acadian redfish) and *S. mentella* (deep-water redfish) are the two most important redfish species and show differential ecological preferences (Atkinson, 1987; Gascon, 2003; Valentin *et al.*, 2006). *Sebastes fasciatus* distribution is mainly restricted to the southern regions (Gulf of Maine, Nova Scotian Shelf), whereas *S. mentella* is distributed northerly. The distribution of the two species overlaps on Flemish Cap, around Newfoundland, and off the Nova Scotian Shelf, but particularly in the Gulf of St. Lawrence and in the Laurentian Channel. The two species are difficult to distinguish in the field and are traditionally managed as a single resource. Although they are morphologically very alike, traditional and geometric morphometric studies have shown that these species are significantly different in term of overall body shape (Valentin *et al.*, 2002, and references therein). The recent microsatellites study (Roques *et al.*, 2001) also gave strong support to a former formulated hypothesis about introgressive hybridization taking place between *S. fasciatus* and *S. mentella* in the

Gulf of St. Lawrence and the Laurentian Channel (Rubec *et al.*, 1991; Desrosiers *et al.*, 1999). Roques *et al.* (2001) clearly demonstrated the contribution of introgressive hybridization in shaping population structure (see also Roques *et al.*, 2002).

Though very valuable, these first studies based on microsatellites only provided an overall description of the redfish population structure in the northwest Atlantic. A finer resolution was needed for the purpose of fishery management. This led to a multidisciplinary research project at the scale of the northwest Atlantic, combining microsatellites with geometric morphometrics. In comparison with the former genetic studies, the project improved the genetic approach by increasing the number and the quality of microsatellite loci (i.e., adding four tetra- and one pentanucleotide microsatellite loci) and by using new statistical methods (e.g., Bayesian statistics). Along with the information regarding population structure (see Chapter 4), the project gave the opportunity to generate valuable information at both the species and the methodological levels, in line with the following objectives. A first objective was to study the implication of the number of microsatellite loci and of locus polymorphism for species discrimination in redfish. A second objective was to further document the pattern of introgressive hybridization between *S. fasciatus* and *S. mentella*, which also led to investigate the possibility of introgressive hybridization involving *S. marinus*. A third objective was to assess the discriminating power and the convergence of the geometric morphometric and genetic approaches, which led to question the hypothesis of morphological convergence caused by introgressive hybridization.

3.3 MATERIAL AND METHODS

3.3.1 Samples

A total of 3211 redfish (representing 57 sets) were collected with a bottom-trawl net, in summer and fall 2001 and 2002. The sampling area comprised NAFO fishing areas from the Gulf of Maine southward to the Labrador Sea northward, and from southwest Greenland. For each set, an average of 60 fishes was frozen immediately on board. Redfish from the Saguenay Fjord were caught using handlines during ice-fishing in winter 2003.

As mentioned above, this study is part of a wider project on redfish population structure in the northwest Atlantic. The issue of population structure shaped the sampling strategy, because this issue required working on samples that were representative of the populations. The sampling strategy was to target large aggregations (more than 100kg by set) of *S. fasciatus* and *S. mentella* that are known to sustain the fishery. *Sebastes marinus* was not targeted and only one sample was taken. It was also important to ensure that each sample was monospecific. Moreover, for sample comparisons, it was important to work with a sample size of at least 30 specimens per sample. Another constraint was the joint use of the morphometric and genetic approaches on the same specimens. All these considerations explain the contrast between the total number of sampled specimens (N = 3211) and the number of specimens finally used in the present study (N = 1121, Table 3.1; see details below for the selection of the specimens).

3.3.2 Sample processing

In the laboratory, the following protocol was used on each of the 3211 specimen. First, samples of liver and muscle tissue were taken on the thawing specimen. The liver sample was immediately frozen, pending electrophoretic analysis of the malate dehydrogenase (MDH) that was carried out later on all specimens (Hebert & Beaton, 1989). The muscle sample was preserved in 100% ethanol. After complete thawing of the specimen, geometric morphometric data consisting of 10 landmarks (L1 to L10) defining the body outline were captured (Figure 3.1). The fish was laid on his right side on a Styrofoam board covered with a wax paper. The position of each landmark was determined by punching a hole in the paper with a needle. A support was used to maintain the needle perpendicular to the board. After landmarks collection, soft anal fin rays (AFC) were counted and the insertion pattern of the gasbladder muscle between ribs (EGM) was recorded along with sex, maturity and length. Finally, each wax paper was numerized. Landmark digitising was carried out using tpsDIG (Rohlf, 2003b): x, y coordinates of the 10 landmarks were determined for each specimen, providing a total of 20 morphometric variables for each individual.

3.3.3 Genetic analyses

Microsatellite analysis was performed on a subset of the specimens (N = 1147) representing 36 sets. The *S. marinus* sample was selected for the analysis, along with 35 sets achieving a good geographical coverage of *S. fasciatus* and *S. mentella* aggregations (Figure 3.2). At this stage, the precise specific composition of each sample was not known, but the three usual species identification criteria (i.e., AFC, EGM, and MDH) gave a good approximation of it. For each of the 36 sets, an average of 32 specimens has been randomly chosen for microsatellite analyses on muscle samples. A total of 13 microsatellite loci were analyzed using fluorescent labelling method. Eight loci (SEB9, SEB25, SEB30, SEB31, SEB33, SEB37, SEB45, and SEB46) were originally characterized for the Atlantic redfish species (Roques *et al.*, 1999a). Five loci were originally isolated from two Pacific redfish species; SAL3 and SAL4 from *S. alutus* (Miller *et al.*, 2000), and SPI4, SPI6, and SPI10 from *S. pinniger* (Gomez-Uchida *et al.*, 2003). The three SPI loci and SAL4 were used extensively for the first time on Atlantic species, while SAL3 has already been used in a study on *S. marinus* (Anonymous, 2004).

DNA was extracted from muscle tissue with DNeasy® Tissue Kit (Quiagen). The 13 microsatellite loci were amplified in three multiplex PCR reactions (MuxI, MuxII, and MuxIII; Table 3.2). Multiplex amplification demanded modification of three primers. Both forward (-F) and reverse (-R) primers for SPI4 and primer-F for SPI10 have been modified in order to yield longer PCR products. These loci are therefore called SPI4II and SPI10II in

the present study. Moreover, the unlabelled SPI6 primer-R competitioned with the unlabelled SPI4II primer-R for amplification at locus SPI4II. After DNA sequence verification, it was confirmed that a site corresponding to primer-R SPI6 was present at locus SPI4II. Therefore, the unlabelled SPI6 primer-R was used to amplify both loci SPI4II and SPI6 (primer-R SPI4II was not used).

Each multiplex reaction was carried out in a 10 µl reaction volume using 2 µL of DNA template (20–50 ng), 50 µM dNTP, 0.245 U of Expand High Fidelity DNA polymerase (Roche), 1× Expand High Fidelity buffer 2 with 1.5 mM $MgCl_2$ (Roche, unknown composition), and 0.15–0.5 µM of each primer. Detailed primer concentrations and PCR parameters are presented in Table 3.2. PCR amplifications were performed with a Robocycler® Gradient 96 temperature cycler equipped with hot top (Stratagene®). For each sample, 1 µL of the PCR product was mixed with 0.1 µL GENESCAN® 400 HD ROX size standard (Applied Biosystems) and 15 µL formamide before denaturation at 95°C for 3 min. Electrophoresis was conducted using an ABI 310 (Applied Biosystems) sequencer, with injection time and runtime set respectively to 5 s and 30 min.

Data analysis and scoring were performed using Genescan and Genotyper software packages (Applied Biosystems). After discarding the 9 specimens having incomplete data, the total sample size was 1138 individuals.

3.3.4 Species assignment

As mentioned above, it was crucial to ensure that each sample was monospecific to avoid that species differences influence sample characteristics within species. Species assignment is challenging for *S. fasciatus* and *S. mentella*. Traditionally, species assignment is based on the MDH, AFC, and EGM criteria that are known to be congruent in absence of introgressive hybridization (*S. mentella*: AFC ≥ 8, EGM between ribs 2 and 3, and *MDH-A*11*; *S. fasciatus*: AFC ≤ 7, EGM between ribs 3 and 4 or more, and *MDH-A*22*; Gascon, 2003 and references therein; Valentin *et al.*, 2006). However, these criteria are inconsistent in presence of introgressive hybridization what makes them unreliable for species identification. Even a codominant marker such as the MDH criterion is inaccurate in such situation because of its low polymorphism. For example, mating between two deeply introgressed *S. fasciatus* specimens, heterozygote at the *MDH-A** locus (*MDH-A*12*), will produce a quarter of homozygote offspring (*MDH-A*11*) that would be wrongly assigned to *S. mentella*. Using a combination of highly polymorphic variables such as microsatellite loci allows more accurate assignment (Roques et al., 1999b).

The microsatellite data for the 1138 specimens representing 36 sets (Table 3.1; Figure 3.2) were submitted to a factorial correspondence analysis (FCA), available in software GENETIX (Belkhir *et al.*, 1996–2004). FCA is an ordination method that projects individuals into a multidimensional space according to their allelic composition. Such analysis does not require setting *a priori* groups. Unsurprisingly, the two first axes revealed

three distinct clusters that were interpreted as representing *S. mentella* and *S. fasciatus* and *S. marinus* (Figure 3.3). The *S. marinus* cluster was easy to identify, because it corresponded to the only *S. marinus* sample available. The three usual criteria (MDH, EGM, and AFC) were examined to decide which cluster corresponded to *S. mentella* and *S. fasciatus*. Despite some discrepancies, it was clear that one cluster was dominated by individuals possessing *S. mentella* characteristics while the other comprised mostly individuals with *S. fasciatus* characteristics. Consequently, the three usual criteria were used to identify the clusters, but they were not used for individual assignment. The specimens were assigned to a given species according to the cluster they belonged to. According to microsatellites, there were 19 *S. fasciatus* samples (N = 596), 16 *S. mentella* samples (N = 495), and one *S. marinus* sample (N = 30). The 17 specimens not belonging to the dominant species of a given sample were discarded from the sample (Figure 3.3), leading to a total sample size of 1121 individuals (Table 3.1).

3.3.5 Statistics on genetic data

3.3.5.1 Sample-based statistics

The data set describing the genotype at the 13 microsatellite loci (36 samples, N_{tot} = 1121) was analyzed with MICRO-CHECKER (Van Oosterhout *et al.*, 2004) to detect scoring incongruities and possible null alleles. The number of private alleles was

determined for each species using the software CONVERT (Glaubitz, 2004). The number of alleles, and allelic richness (A) were calculated over loci for the 36 samples, using the software FSTAT (Goudet, 2001). Observed (H_o) and expected (H_e) heterozygosities over loci were computed for each sample using the software GENETIX (Belkhir *et al.*, 1996–2004). The software FSTAT (Goudet, 2001) was used to estimate F_{IS} (i.e., the heterozygote deficit within samples) over loci, for each species, and to estimate F_{IT} (i.e., the global heterozygote deficit) over loci, within each species. F_{IS} and F_{IT} were tested (1 000 randomizations) for significant differences from zero to assess the compliance with the Hardy-Weinberg equilibrium. All probability values were adjusted for multiple comparison tests using sequential Bonferroni adjustments (Rice, 1989). The mean values of A, H_o and H_e were compared between the *S. fasciatus* and the *S. mentella* samples using the Mann-Whitney non-parametric test.

Genetic differentiation between pairs of the 36 samples was quantified by estimates of pairwise fixation indices (F_{ST}) based on variance in allelic frequencies according to Weir & Cockerham (1984) available in ARLEQUIN (Schneider *et al.*, 2000). The computations were based on allelic frequencies, because several loci did not follow stepwise mutational model. Test of pairwise differences for F_{ST} values were computed using 100 000 permutations with probabilities values adjusted for multiple comparisons, using Bonferroni sequential correction (Rice, 1989). The mean values for pairwise F_{ST} between samples were calculated for each pair of species.

Hierarchical analysis of molecular variance (AMOVA) was used to partition genetic diversity among the three species, and among and within samples using software ARLEQUIN (Schneider *et al.*, 2000). The procedure was then repeated locus-by-locus. The computations were based on allelic frequencies, because several loci did not follow stepwise mutational model. Number of permutations was set to 10 000 for statistical tests. The among-group (here species) fixation index is called F_{CT} in ARLEQUIN (Schneider *et al.*, 2000). The notation F_{ST} will be used to refer to this estimate.

In order to explore the implication of locus polymorphism for species discrimination, the single-locus F_{ST} values among species were plotted against the single-locus H_S values. The theoretical maximal single-locus F_{ST} values ($F_{ST}max$) were computed, as 1-H_S (Hedrick, 1999), and plotted on the same graph. The single-locus G'_{ST} values (Hedrick, 2005) were also computed and plotted on the same graph. G'_{ST} is a measure of genetic differentiation, which is standardized for the level of polymorphism, allowing direct comparison between loci.

3.3.5.2 Individual-based statistics

The extent of genetic divergence among pairs of individuals was quantified by Cavalli-Sforza & Edwards (1967) chord distance (D_{CE}) using software POPULATIONS (Langella, 1999). This matrix of inter-individual genetic distances was used to test for

independence between the genetic and morphometric data sets (see Mantel test described below).

The program STRUCTURE (Pritchard *et al.*, 2000; Falush *et al.*, 2003) was run on the total data set (N = 1121) to detect admixed specimens between the three species. This program implements a model-based clustering method for inferring population structure, along with assigning individuals to populations, and identifying migrants and admixed individuals. It is assumed that the loci within populations are at Hardy-Weinberg equilibrium, and linkage equilibrium. The individuals are assigned to populations on a probabilistic basis in such way as to achieve these assumptions. For each individual, the program computes the proportion of the genome originating from each population, and displays the results graphically. When unknown, the number of populations (K) is also inferred on a probabilistic basis. HW disequilibrium associated with strong deficit in heterozygote was detected in both *S. fasciatus* and *S. mentella* when all samples were pooled by species. This reflected the presence of population structure within species (Wahlund effect) that was further confirmed by the pairwise F_{ST} values between samples within species (see Chapter 4). For this reason, setting K = 3 was not appropriate, because the assumptions of the program were not met. It was assumed that *S. fasciatus* and *S. mentella* would each be characterized by a different group of populations, and *S. marinus* by one population. K was estimated using three simulations per K, with K varying from 3 to 12. The parameters were a burn-in period of 200 000 steps followed by 200 000 Markov chain Monte Carlo (MCMC) repetitions under the correlated model. The results indicated

that these simulation parameters were appropriate to detect admixed specimens between species.

3.3.6 Statistics on geometric morphometric data

All statistical procedures on morphometric data have first been performed separately for males and females, because sexual dimorphism has been reported for North Atlantic redfish species (Valentin *et al.*, 2002 and references therein). These separated analyses showed convergent results regarding the issue of species discrimination addressed in the present study (not shown, but see Chapter 4). Therefore, the data were pooled and analyzed together.

Morphometric data of the 1121 specimens representing the 36 samples have been submitted to a *generalized Procrustes analysis* (GPA) using software tps RELATIVE WARPS (Rohlf, 2003c). This procedure translated, rotated and scaled (to unit centroid size) the original configurations in order to achieve the best superimposition of all shapes. The new coordinates, called superimposed coordinates, have been corrected for upward/downward arching artefact (Valentin *et al.* 2003; Chapter 2).

The matrix of Euclidian inter-individual distances was computed on the corrected superimposed coordinates. The correlation between this matrix and the matrix of inter-

individual D_{CE} genetic distances was tested through a Mantel test with 1 000 permutations using software NTSYSpc (Rohlf, 2002). This was done to test for the concordance between the two data sets.

The corrected coordinates have been transformed into shape variables called partial warps (procedure available in the software tps RELATIVE WARPS, Rohlf, 2003c). The partial warp scores were introduced in a discriminant function analysis (DFA) using the statistical package Systat (©2002, SYSTAT Software Inc.). These scores were thus combined in two linear functions that best discriminated individuals of the three species (as defined by microsatellites) on the basis of their body shape. A cross-validation procedure was used to assess the classification efficiency obtained by the discriminant functions. This consisted in computing the two functions with only two thirds of the specimens, and in estimating the classification success for the last third of the specimens. The individual discriminant scores were plotted on the same graphic for the three species. Specimens that were the most genetically admixed were marked on the graphic to illustrate how they scored in comparison with the "pure" specimens. An intermediate position would indicate morphological convergence caused by introgressive hybridization. Shape variations associated with the discriminant functions were visualized with tpsREGR (Rohlf, 2000) using the vector of DFA scores as the independent variable.

3.4 RESULTS

3.4.1 Descriptive statistics

A summary of the descriptive standard statistics—number of alleles, number of private alleles, allelic richness (A), observed (H_o) and expected (H_e) heterozygosities, and tests for deviation from Hardy-Weinberg proportions (HWE)—is given in Table 3.3. The results are summarized by species. The details for the 36 samples, including the results of the MICRO-CHECKER analysis, are presented in Chapter 4, which focuses on population structure within species.

The total number of alleles reached 375 (Table 3.3). When data were pooled by species, 106 private alleles were found, with 7.7%, 2.4%, and 18.1% of the total number of alleles being private to *S. fasciatus*, *S. marinus*, and *S. mentella*, respectively. Most private alleles were present at low frequency (< 1%) within each species. Two significant exceptions were alleles SEB31-183 and SEB37-231 for *S. mentella*, which frequencies reached 9.1% and 21.5%, respectively.

Genetic variability was high within samples, with allelic richness (A) across loci ranging from 102.8 to 164.6 (mean = 143.6). Allelic richness was significantly higher for *S. mentella* samples than for *S. fasciatus* ones ($P < 0.00001$, Chi^2 approx. = 23.1), with A values ranging from 146.2 to 164.6 (mean = 155.6), and 102.8 to 153.3 (mean = 133.2),

respectively. For the single *S. marinus* sample, *A* was closer to *S. mentella* mean value (149.9). Allelic richness per locus across samples varied between 5.2 (SAL3) and 21.8 (SEB30) (mean = 13.1). Allelic polymorphism was lower for SAL loci than for SEB and SPI loci.

Overall heterozygosity was high with H_o values ranging from 0.673 to 0.817 (mean = 0.745). Heterozygosity was significantly higher for *S. mentella* samples relative to the *S. fasciatus* ones ($P = 0.0016$, Chi^2 approx. = 9.9), with H_o values ranging from 0.695 to 0.817 (mean = 0.765), and 0.673 to 0.757 (mean = 0.727), respectively. For the *S. marinus* sample, H_o (0.790) was closer to *S. mentella* mean value. No departure of HWE was observed in the *S. marinus* sample, but a significant deficit in heterozygotes was observed within *S. mentella* (pooled samples), and within *S. fasciatus* (pooled samples) (Table 3.3).

3.4.2 Genetic differentiation and AMOVA

Pairwise F_{ST} values between samples among species were all highly significant ($P < 0.00001$), averaging 0.129 between *S. mentella* and *S. fasciatus*, 0.091 between *S. mentella* and *S. marinus*, and 0.131 between *S. fasciatus* and *S. marinus*. Most of the genetic variation (86.3%) was explained by differences within samples. Interspecific differences accounted for 12.8% of the total variance, while variation among populations

within species represented only 0.86% of the total variance. Differences among species, and among populations within species were highly significant ($P < 0.00001$).

Locus-by-locus AMOVA indicated that differences among species were highly significant ($P < 0.00001$) at all 13 loci, with F_{ST} values ranging from 0.032 to 0.336, suggesting a genome-wide differentiation (Table 3.4). Single-locus F_{ST} values were negatively correlated (r_S = -0.92; $P < 0.00001$) with the level of locus polymorphism, expressed as H_S (i.e., the mean within-population observed heterozygosity; Table 3.4, Figure 3.4). The less polymorphic loci (SAL4, SAL3, and SEB31) presented F_{ST} values over 0.245, while the values for the more polymorphic loci (SEB37, SPI4II, and SEB33) did not exceed 0.051. G'_{ST} values were maximal for SEB30 and SEB31, two loci with different levels of polymorphism (Table 3.4, Figure 3.4). SPI10II exhibited the highest G'_{ST} values among the new set of loci (i.e., SAL and SPI). Overall, the most polymorphic loci (H_S over 0.7) reached highest G'_{ST} values than less polymorphic loci, but there was no significant correlation between G'_{ST} and H_S values.

3.4.3 Analysis with the program STRUCTURE

The highest posterior probability ($P \cong 1$) was reached with K=8, for the analysis performed on the total data set comprising redfish from the three species. These eight most probable populations clustered in three well defined groups representing the three species

(Figure 3.5). The genetic make-up of each specimen was clearly dominated by one species, as illustrated by mean values of the proportion of individual membership to *S. fasciatus* (0.97±0.05), *S. mentella* (0.92±0.07), and *S. marinus* (0.94±0.04) calculated within each corresponding group.

For each species, the presence of genetic background originating from the two other species was observed in each sample (Table 3.5, Figure 3.5). Considering the common ancestry of the three species, finding shared polymorphism was not unexpected (Sundt and Johansen, 1998; Kendall, 2000, and references therein). The background could also reflect some limitation of the analysis for discriminating the species. Therefore, no inferences will be drawn from the absolute values provided by the STRUCTURE analysis, and the interpretation of the results will be based on the relative values, in a descriptive manner. Regarding the admixture between the two beaked redfish species, *S. mentella* was more influenced by *S. fasciatus*, than *S. fasciatus* was by *S. mentella* (Table 3.5). The degree of individual admixture varied between 1.0% and 36.6% (mean = 5.8%, SD = 5.9) within *S. mentella*, and between 0.4% and 36.8% (mean = 1.8%, SD = 3.6) within *S. fasciatus* (Figure 3.5). For *S. fasciatus*, four samples (3PS114, 3PS88b, 4R107, and 4VN67) from the Gulf of St. Lawrence–Laurentian Channel (GSL–LCH) area showed increased genetic background originating from *S. mentella*. Three of these samples (3PS114, 3PS88b, and 4VN67) also presented increased genetic background originating from *S. marinus*, with value of admixture exceeding 10% for ten specimens. For *S. mentella*, samples from the northeast (NE) area, located outside the GSL–LCH region, exhibited lower genetic

background originating from *S. fasciatus* than samples from the GSL–LCH area. One sample (2J42) from the NE-outside-GSL–LCH region was the most influenced by genetic background originating from *S. marinus*, followed by several samples from the GSL–LCH area. Two *S. mentella* specimens (from samples 2J42, and 4R51) exhibited more than 43% of admixture originating from *S. marinus*.

The pattern of admixture observed between *S. fasciatus* and *S. mentella* was in agreement with the hypothesis of introgressive hybridization occurring between *S. fasciatus* and *S. mentella* in the GSL–LCH area (Roques *et al.*, 2001). Assuming this hypothesis, it was possible to distinguish the amount of shared ancestral polymorphism from introgression by comparing the level of genetic background from the alternative species, between the specimens from inside, and outside the GSL–LCH area (Table 3.5). Following this strategy, the level of introgression was estimated at 3.5% (i.e., 6.4-2.9) for *S. mentella*, and 1.4% (i.e., 2.6-1.2) for *S. fasciatus*.

3.4.5 Geometric morphometrics

The Mantel test performed between the matrices of inter-individual genetic and morphometric distances indicated that the two matrices were not independent (r_S = 0.041, P = 0.001). This suggested an overall, though weak, concordance between the genetic and the morphometric data sets, at the individual level. The concordance was much more

important when individuals were pooled by species, as suggested by the discriminant analysis. Indeed, the DFA performed on the geometric morphometric data (partial warp scores) was successful to discriminate the three species defined *a priori* on the basis of microsatellite data ($P < 0.0001$, Approx. $F = 120.7$, df = 30, 2208). The first axis summarized 93.8% of the total dispersion and allowed a clear discrimination between *S. fasciatus* and *S. mentella* (Figure 3.6). *Sebastes marinus* was mostly differentiated on the second axis and laid between the other two species on the first axis, but slightly closer to *S. fasciatus*. The two discriminant functions were successful in assigning 95% of the specimens to their respective species. This value held for the cross-validation analysis, where one third of the specimens were left out for the function computation and assigned *a posteriori*. Only one *S. fasciatus* specimen was clearly wrongly classified as *S. mentella* by the DFA. This specimen is hardly distinguishable on Figure 3.6, but its coordinates on the discriminant axis are (-2.4; -0.3). All other misclassified specimens were located at intermediate position between the clusters. These misclassified specimens did not correspond to the most genetically admixed specimens and, consequently, the most admixed specimens clustered mostly within the three clusters representing the species (Figure 3.6).

3.5 DISCUSSION

3.5.1 Power of loci for species discrimination in relation with locus polymorphism

Using 13 loci has improved the power of discrimination between *S. fasciatus* and S. *mentella*. Comparing graphical results of a FCA performed on the eight original SEB loci (not shown) with the results of the same analysis incorporating the three SPI loci and the two SAL loci (Figure 3.3) showed a clear improvement of the species discrimination. This improvement had little repercussions on the value of F_{ST} which raised from 0.127 with 8 loci to 0.129 with 13 loci. Using the 8 SEB loci, Roques *et al.* (2001) reported a mean F_{ST} value of 0.103. This discrepancy may be related to the way specimens were selected. In the present study, the FCA analysis was used to assign the specimens to their respective species before computation of the F_{ST}. In contrast, Roques *et al.* (2001) assigned the specimens according the 3 usual criteria (MDH, AFC, EGM). This may have led to some misclassification between the two species, and the misclassified specimens contributed to lower the F_{ST} value.

According to Roques *et al.* (1999b), loci SEB9 and SEB31, along with SEB 25 and SEB30 formed the best combination of four loci to allow a correct classification of the specimens to their respective species (considering the four Atlantic redfish species). In the present study, four loci (SEB9, SEB31, SAL3, and SAL4) were the most different between the species, based on the pairwise fixation indices F_{ST}. Besides, the locus-by-locus

AMOVA indicated that F_{ST} values (the among-species component of variance) for SAL3 and SAL4 exceeded by more than three times those for SEB25 and SEB30. At first, these results would suggest that SAL3 and SAL4 were mostly responsible for the improvement of the discrimination between species. However, such interpretation is simplistic, because it does not consider the amount of locus polymorphism, which has a direct influence on the F_{ST} values. Indeed, SAL3 and SAL4 were the least polymorphic loci. Figure 3.4 clearly illustrated that no loci other than SEB31 could have reached the high F_{ST} values presented by SAL3 and SAL4. When the amount of species differentiation was expressed as G'_{ST} values, it confirmed the results of Roques *et al.* (1999b) regarding the importance of SEB30 and SEB31 for species discrimination. SEB25 was less discriminant than previously observed (Roques *et al.*, 1999b), a discrepancy that could be related to the presence of *S. viviparus* in that study. In summary, the additional set of loci (i.e., SAL and SPI) used in the present study significantly contributed to the species discrimination, although only SPI10II performed as well as the SEB loci, according to the G'_{ST} values.

3.5.2 Homoplasy

It has been proposed that inverse relationship between locus polymorphism and F_{ST} estimates may be a signal of high mutation rate favouring size homoplasy (SH) at the most polymorphic loci (O'Reilly *et al.*, 2004). Size homoplasy occurs when alleles at a locus are identical in state, but not identical by descent, which leads to underestimate the level of

differentiation. In the present study, Figure 3.4 clearly indicated that the level of locus polymorphism *per se*, rather than SH, was the main factor constraining the F_{ST} values. Besides, the G'_{ST} values indicated that species differentiation was not constrained by the level of polymorphism. However, it cannot be ruled out that different rate of SH at the different loci influenced the estimates of population differentiation. Indeed, loci SEB30 [$(CT)_nTT(CT)_nTT(CT)_n$] and SEB31 [$(GA)_nGGGG(GA)_nGGG(GA)_n$], which exhibited the highest G'_{ST} values, were the only two interrupted microsatellites. Interrupted microsatellites are thought to present lower rate of SH. This characteristic could explain the good performance of loci SEB 30 and SEB31 relative to the uninterrupted loci (e.g., loci SEB25, SEB9, and SEB45) exhibiting comparable amount of polymorphism (Estoup *et al.*, 2002, and references therein).

3.5.3 Introgressive hybridization between *S. mentella* and *S. fasciatus*

The large amount of polymorphism for microsatellite markers makes possible to analyse contemporary gene flow using individual assignment methods. Simulations studies have shown that homoplasious mutations affect the efficiency of assignment methods (Cornuet *et al.*, 1999), which could have influenced the results produced by the program STRUCTURE. However, the most variable markers produce better assignment scores than less polymorphic ones for a given value of F_{ST}, even though high levels of polymorphism are slightly less informative when F_{ST} is high (e.g., the present study) than when it is low

(Estoup *et al.*, 2002; Kalinowski, 2004). Besides, Monte Carlo Markov chain method, which is implemented in the program STRUCTURE, is among the best performing approach for estimating admixture proportion using microsatellites (Choisy *et al.*, 2004).

The results of the analysis performed with the program STRUCTURE gave an elegant confirmation of the presence of bidirectional introgressive hybridization taking place between *S. mentella* and *S. fasciatus*. The rates of introgression for *S. mentella* (3.5%) and *S. fasciatus* (1.0%) were much lower than those found by Roques *et al.* (2001) who reported a mean value of 17.9% and 11.3%, respectively. A strict comparison between the values of the two studies was not recommended because these values were estimated using different methods and different number of loci. However, the higher introgression rate in *S. mentella*, relative to *S. fasciatus*, was confirmed. It can be assumed that hybrids between these two species were mostly backcrosses, since no individual exceeded 37% of admixture (keeping in mind that this absolute value should not be interpreted too literally, without information about the power of the analysis). This situation was interpreted by Roques *et al.*, (2001) as a sign of rare hybridization, and/or of numerous backcrosses between F1 and the parental type. Different levels of introgression were observed within all size-class (not shown), suggesting that hybridization was not related to a particular cohort. The results also confirmed that introgressive hybridization between *S. mentella* and *S. fasciatus* was mostly restricted to the GSL–LCH area. However, an introgressed specimen was observed in the Gulf of Maine (see sample s266 from the SW-outside-GSL–LCH region), which is an allopatric zone for *S. fasciatus*. It is worth mentioning that admixture with *S. marinus* was

also observed in the s266 sample. These observations suggest that introgressive hybridization taking place in the GSL–LCH area may have genetic repercussion in other areas, through migration of introgressed specimens. Based on *S. mentella* samples, Roques *et al.* (2001) have shown that introgressive hybridization was very limited between the two species in the area of the Grand Bank and in Labrador Sea (i.e., the NE-outside-GSL–LCH region). The present study was consistent with these former results, showing little sign of introgressive hybridization in both *S. mentella* and *S. fasciatus* samples from the NE-outside-GSL–LCH region. This pattern was also consistent with the overall congruence of the three usual criteria (MDH, AFC, EGM) observed in the 3LNO NAFO Divisions for the two species (Gascon, 2003; Valentin *et al.*, 2006). These observations suggest that the conditions for introgressive hybridization to occur are not met in the 3LNO area, even though the two species are in sympatry. The situation could be different on the Flemish Cap, where the congruence between the three criteria is known to be lower and where heterozygote specimens at the *MDH-A** locus are less rare (Gascon, 2003). Unfortunately, no data from the Flemish Cap area were available for the present study. However, the graphical results (generated with the program STRUCTURE) of a recent study analyzing microsatellite data for redfish from Flemish Cap suggested that introgressive hybridization may take place in this area (Anonymous, 2004, p.216).

3.5.4 Revisiting the hypothesis of morphological convergence

Many hypotheses have been proposed to explain the restriction of introgressive hybridization inside the GSL–LCH area—intermittent period of spatial overlap, numerical imbalance, environmental characteristics, and incomplete reproductive barriers (see Roques *et al.*, 2001 for a complete discussion). These hypotheses still hold for the present study. The results brought additional insights regarding the hypothesis of incomplete reproductive barriers associated with morphological convergence in sympatry. These viviparous species are expected to undergo assortive mating, and it was proposed that interspecific mating could occur because of size and morphological convergence in sympatry (Roques *et al.*, 2001). The present study did not support this hypothesis, since there was no difference in the assignment success between two DFA performed respectively on samples from inside and outside the GSL–LCH area (not shown). In contrast, Valentin *et al.* (2002) supported the hypothesis of morphological convergence in the GSL. Their DFA based on geometric morphometric was less successful in assigning specimens to their correct species than a comparable analysis performed on redfish from the Grand Bank area (Saborido-Rey, 1994). The discrepancy between the two Valentin *et al.* studies lies in the fact that the assignment criterion was different. Valentin *et al.* (2002) used the genotype at the malate dehydrogenase locus (*MDH-A**) instead of microsatellites. The genotype at the *MDH-A** locus is not reliable to discriminate the species inside the GSL–LCH area, because of its low polymorphism (only two alleles), that cannot reflect the level of genetic admixture caused by introgression. In this area, the genotype at the *MDH-A** locus was not congruent

with the microsatellite data in more than 13% of the specimens (not shown). When the genotype at the *MDH-A** locus was used instead of the microsatellites in the DFA, the success of classification lost 15%. So, morphological convergence, at least for body shape, is not observed in the sympatric area, and, therefore, is not a factor favouring introgressive hybridization between *S. mentella* and *S. fasciatus*. In fact, considering the small (though significant) amount of shape differences observed between species, reproductive barriers through mate recognition are probably not based on body shape. Mate recognition could depend on other characteristics such as courtship behaviour, which has been documented for related species (Helvey, 1982; Shinomiya & Ezaki, 1991; Gingras *et al.*, 1998), or on chemical communication (e.g., chemical signals responsible for mate selection according to its haplotype at the major histocompatibility complex; Rosenthal & Lobel, 2005; and references therein).

Reciprocally, introgressive hybridization did not seem to promote morphological convergence. This is supported by the observation that the most admixed specimens clustered mostly within the three clusters representing the species (Figure 3.6). The relationship between introgressive hybridization and morphology has already been discussed in ontogenetic and evolutionary perspectives (Valentin *et al.*, 2002). It was discussed whether introgressive hybridization could generate greater morphological variability in hybrids, that would reflect developmental instability resulting from the rupture of co-adapted parental gene complexes through hybridization (Neff & Smith, 1979; Strauss, 1986). The present study gave no evidence of higher morphological variability in

the most genetically admixed specimens in comparison with the "pure" specimens (according to the scores of these specimens on the discriminant function). This observation could be an indication that selection is acting. Unfortunately, an attempt to document fluctuating asymmetry (FA), which is considered to reflect developmental instability (Polak, 2003), was unsuccessful (unpublished data). The failure was caused, in part, by the difficulty to find appropriate variables, meeting the assumptions of a FA study (Palmer & Strobeck, 2003).

3.5.5 Discriminating power: microsatellites versus geometric morphometrics

Genetic factors were definitely involved in the determination of redfish body shape. Interspecific shape differences were maintained across a large geographical area undergoing various environmental conditions. The matrices of morphometric and genetic distances were significantly correlated, and the discriminant function based on morphometric data reached a classification success of 95% (for the specimens assigned *a priori* to species with microsatellites). This allows considering geometric morphometrics as an inexpensive alternative to microsatellites for species assignment, at least between *S. fasciatus* and *S. mentella* (the *S. marinus* sample was too small to allow strong conclusions). However, an assignment procedure based on morphometrics still requires some genetic information in order to calibrate the morphometric variables. Such calibration is important to get rid of the non informative variability which could lower the discriminant

power. For example, it has been shown that measurement error generated by slight difference in the way the fish is laid flat during data capture generates shape variability that is larger than interspecific shape differences (see Chapter 2). Calibrating the discriminant model can be achieved by partitioning the morphometric variance using regressions (Berge & Penin, 2004).

3.5.6 Introgressive hybridization involving S. marinus

If the pattern observed between *S. mentella* and *S. fasciatus* is interpreted as bidirectional introgressive hybridization, it must be considered that introgressive hybridization also takes place between *S. marinus* and the two beaked redfish species. The introgression appeared as bidirectional between *S. marinus* and *S. fasciatus*. Around 10 *S. fasciatus* specimens showed clear sign of introgression from *S. marinus*, but the reciprocal was less obvious (probably due to the small sample size for *S. marinus*). Additional evidence of introgression from *S. fasciatus* towards *S. marinus* came from an analysis of the same data, but incorporating one *S. marinus* sample from Norway, an area where *S. fasciatus* is absent. The analysis was performed with the eight SEB loci. The results have shown a genetic background originating from *S. fasciatus* in the *S. marinus* samples from both the GSL–LCH area and Norway. This observation is easily explained by the common ancestry of the species, some level of microsatellite homoplasy, and maybe some limitation in the power of the analysis. Nevertheless, the level of admixture was significantly (P =

0.033) higher in the GSL–LCH region (3.9%) than in Norway (1.7%) according to a Kruskal-Wallis comparison test. The analysis of the variation at the mitochondrial gene ND3, and at the *MDH-A** locus suggested occurrence of introgressive hybridization between the two species on the Flemish Cap, and in the GSL (Johansen, 2003; Anonymous, 2004, p227).

Regarding *S. marinus* and *S. mentella*, the present study provided evidence of introgression towards *S. mentella*. Besides, the two *S. mentella* specimens exhibiting more than 43% of admixture with *S. marinus* (samples 2J42 and 4R51, Figure 3.5) could hypothetically represent F1 specimens, which would suggest an ongoing introgressive hybridization. Introgression from *S. mentella* towards *S. marinus* was not observed, but the sample size was small. The graphical results (generated with the program STRUCTURE) of a recent study analyzing microsatellites and amplified fragment length polymorphism (AFLP) were consistent with the hypothesis of introgressive hybridization taking place between the two species on the Flemish Cap (Anonymous, 2004).

Hybridization involving *S. marinus* would imply that reproductive barriers with the two other species are low. The maturity data gathered in the present study should only be considered as indicative, because they were determined visually on thawed specimens. However, they indicated no significant differences between the three species regarding the timing of reproduction in the GSL–LCH area (not shown). This is in line with a former study on reproductive characteristics of *S. mentella* and *S. fasciatus* that suggested no

strong reproductive barriers between these two species in the Gulf of St. Lawrence (St-Pierre & de Lafontaine, 1995). On the Flemish Cap, some overlapping in the period of larval release was observed for the three species (Saborido-Rey, 1994). It has however to be mentioned that the critical period for hybridization to occur is copulation. The period of copulation is difficult to infer from maturity data, because the females are known to store the sperm for several months, before internal fertilization occurs (Saborido-Rey, 1994 and references therein).

The possibility of hybridization involving *S. marinus* has always been formulated diffidently. This reticence may reflect the fact that *S. marinus* was considered as of little commercial importance and therefore neglected. Another explanation could be that the issue of hybridization involving *S. marinus* was mostly a by-product of studies dealing primarily with population structure. The reticence in recognizing hybridization could also be related to the zoologist premiss which (in line with Mayr's *biological species concept*) considers species as reproductively isolated groups (Mallet, 2005). Indeed hybridization may be rare on an individual basis. However, there is rising evidence that hybridization and introgression are not rare at the species level, and that the variation introduced by way of introgression regularly contributes to adaptation and diversification (Arnold, 1997; Mallet, 2005).

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Table 3.1 Description of the samples: date, fishing zone, geographical location, depth (m), and mean length (cm) by sex. Each sample is monospecific based on microsatellites data (17 specimens discarded), and do not comprises specimens with incomplete genetic data (9 specimens discarded), so that $N_{TOT} = 1121$ (instead of 1147, initially).

							Males			Females			TOT
Sample	Date	NAFO	Longitude	Latitude	Depth	Mean	SD	*N*	Mean	SD	*N*	*N*
Name		Div.	(W)	(N)	(m)	Size (cm)			Size (cm)			
S. fasciatus												
Northeast, outside GSL–LCH												
3L65	fall 2001	3L	47°16.6	46°51.8	404	20.9	1.2	17	22.9	3.7	7	**24**
3N23	fall 2001	3N	50°22.2	42°47.5	408	23.5	2.4	14	26.1	3.5	18	**32**
3O44	fall 2001	3O	52°56.0	44°11.6	408	20.4	0.9	18	20.8	1.2	14	**32**
3PS1	summer 2002	3PS	55°10.2	45°08.2	202	24.7	1.5	16	26.3	2.2	16	**32**
3PS138	fall 2002	3PS	56°01.8	44°51.9	448	24.4	2.8	8	27.3	2.3	24	**32**
3PS26	summer 2002	3PS	56°14.4	44°52.2	354	22.1	1.2	17	25.6	2.9	15	**32**
Inside GSL–LCH												
3PS114	summer 2002	3PS	56°50.2	47°19.4	241	26.0	5.1	9	29.1	6.7	23	**32**
3PS88b	fall 2002	3PS	57°27.3	47°07.6	234	27.5	2.2	15	30.1	4.0	14	**29**
4R107	summer 2001	4R	59°10.8	49°45.5	217	26.8	1.5	18	30.6	1.9	13	**31**
4VN67	summer 2002	4VN	58°19.7	45°55.0	219	26.1	2.8	14	31.6	3.2	18	**32**
4VS36	summer 2002	4VS	57°42.2	45°16.3	217	25.1	1.2	12	27.3	2.5	20	**32**
4R53	fall 2002	4R	59°44.3	48°17.8	248	23.7	1.6	12	26.6	2.4	20	**32**
4VN5	fall 2002	4VN	58°07.0	45°47.1	245	24.9	1.5	6	28.9	2.4	26	**32**
BonBay	spring 2002	4R	—	—	—	24.1	1.4	9	29.0	3.3	23	**32**
Southwest, outside GSL–LCH												
NS85	summer 2001	4X	65°19.5	43°00.0	153	22.7	2.0	10	25.3	3.7	22	**32**
NS95	summer 2001	4W	60°03.2	43°27.4	504	27.5	1.3	7	34.1	2.9	25	**32**
s261	fall 2001	5Z	67°04.1	42°18.9	297	25.0	1.5	7	30.5	3.9	25	**32**
s266	fall 2002	5Z	69°54.3	42°18.7	203	22.3	1.1	20	26.3	1.6	12	**32**
s327	fall 2001	5Y	67°17.7	43°49.0	197	22.3	0.8	16	25.4	2.4	16	**32**
								245			**351**	**596**

Table 3.1 (continued)

Sample Name	Date	NAFO Div.	Longitude (W)	Latitude (N)	Depth (m)	Males Mean Size (cm)	Males SD	Males *N*	Females Mean Size (cm)	Females SD	Females *N*	TOT *N*
S. mentella												
Northeast, outside GSL–LCH												
2J42	fall 2001	2J	53°15.8	54°33.7	671	26.1	2.4	12	26.2	2.5	19	**31**
3L29	fall 2001	3L	47°25.4	48°03.1	506	24.4	2.1	10	24.9	1.7	18	**28**
s1050	fall 2001	1F	47°09.5	60°09.2	357	21.3	1.3	15	21.3	1.6	16	**31**
Inside GSL–LCH												
3PN1	summer 2002	3PN	58°50.0	46°53.3	434	29.1	1.5	21	30.2	2.1	11	**32**
3PN77	fall 2002	3PN	58°24.0	47°08.3	390	28.1	1.8	13	29.7	1.4	19	**32**
3PS133	fall 2002	3PS	56°49.2	45°32.0	394	29.3	2.8	13	30.8	1.8	18	**31**
4R48	summer 2002	4R	59°46.0	48°07.3	315	27.6	1.2	11	30.0	3.9	18	**29**
4R51	fall 2002	4R	59°58.9	48°19.1	392	30.3	1.4	18	32.7	1.0	14	**32**
4S35	fall 2002	4S	60°50.5	48°19.5	429	30.0	2.0	17	33.3	1.4	14	**31**
4S44	summer 2002	4S	60°34.2	48°03.6	447	29.7	2.2	15	31.4	2.0	16	**31**
4VN12	fall 2002	4VN	59°37.2	46°58.3	431	29.6	2.2	19	32.1	2.1	12	**31**
4VN2	fall 2002	4VN	57°53.0	45°42.8	425	29.3	1.9	15	31.7	1.5	17	**32**
4VN77	summer 2002	4VN	58°55.4	46°22.8	348	29.3	1.9	16	30.2	1.7	16	**32**
4VS13	summer 2002	4VS	58°02.8	44°13.8	515	30.0	1.6	15	31.7	1.6	15	**30**
4VS147	fall 2002	4VS	57°07.9	44°46.3	420	28.9	2.7	12	31.4	1.8	19	**31**
sag	winter 2003	SAG	—	—	—	25.5	0.8	19	26.0	1.1	12	**31**
								241			**254**	**495**
S. marinus												
3PN73	fall 2002	3PN	58°51.8	47°24.9	203	38.4	3.3	**19**	45.8	2.0	**11**	**30**
TOTAL								**505**			**616**	**1121**

Table 3.2 Primer concentrations, fluorescent labels (Applied Biosystems), and PCR parameters for the three multiplex reactions (MuxI, MuxII, MuxIII) used for amplification of the 13 microsatellite loci. The unlabelled SPI6 primer-R was used to amplify both loci SPI4II and SPI6.

	Primer concentrations (µM)			PCR parameters			
	Forward	Reverse	Label		Time (s)	Temp (°C)	Nb cycles
MuxI							
SEB9	0.20	0.20*	6-FAM	Initial Denat.	135	95	× 1
SEB25	0.25	0.25*	NED	Denaturation	30	94	
SEB31	0.30	0.30*	VIC	Annealing	25	55	× 30
SEB33	0.30	0.30*	6-FAM	Extension	25	72	
				Final ext.	180	72	× 1
MuxII							
SAL4	0.30	0.20*	6-FAM	Initial Denat.	135	95	× 1
SEB30	0.30	0.25*	NED	Denaturation	30	94	
SEB37	0.25	0.30*	6-FAM	Annealing	25	52	× 30
SEB46	0.20	0.30*	VIC	Extension	25	72	
				Final ext.	180	72	× 1
MuxIII							
SEB45	0.20	0.20*	6-FAM	Initial Denat.	180	94	× 1
SAL3	0.20	0.20*	NED	Denaturation	30	94	
SPI4II	[a]0.30*	—	VIC	Annealing	30	54	× 30
SPI6	0.15*	0.25	VIC	Extension	30	72	
SPI10II	[b]0.50*	0.50	NED	Final ext.	180	72	× 1

* indicates fluorescence

[a] sequence: 5'-GTGTTATTTGTTGAGACATTAGCTG-3' (different from Gomez-Uchida *et al.*, 2003)

[b] sequence: 5'-ATGATTGGCCAGTATTATTATTTAACA-3' (different from Gomez-Uchida *et al.*, 2003)

Table 3.3 Descriptive statistics of microsatellite data summarized by species. Number of alleles, allelic richness (A), and observed (H_o) and expected (H_e) heterozygosities are values over loci, averaged over samples (ranges of values are in parentheses). $P_{(F_{IS})}$ is the probability of heterozygote deficit over loci within samples. $P_{(F_{IT})}$ is the probability of the global heterozygote deficit over loci within species. Significant values are in bold characters.

	S. fasciatus	*S. mentella*	*S. marinus*	**Total**
Nb of samples	19	16	1	36
Nb of individuals	596	495	30	1121
Nb of private alleles	29	68	9	106
Mean within-sample nb of alleles	145.4 (114.0–165.0)	169.8 (160.0–179.0)	162	375[a]
Mean within-sample A (N = 24)	133.2 (102.8–153.3)	155.6 (146.2–164.6)	149.9	—
Mean within-sample H_o	0.727 (0.673–0.757)	0.765 (0.695–0.817)	0.790	—
Mean within-sample H_e	0.759 (0.689–0.787)	0.801 (0.765–0.822)	0.799	—
F_{IS} (within samples)	**0.044**	**0.045**	0.011	—
$P_{(F_{IS})}$	< 0.001	< 0.001	0.316	—
F_{IT} (within species)	**0.054**	**0.053**	0.011	—
$P_{(F_{IT})}$	< 0.001	< 0.001	0.316	—

[a] total number of alleles

Table 3.4 Heterozygosity and differentiation statistics by locus: mean within-sample heterozygosity (H_S), fixation index between species (F_{ST}), maximum theoretical fixation index between species ($F_{ST}max$), and fixation index between species standardized for locus polymorphism (G'_{ST}; Hedrick, 2005).

Locus	H_S[a]	F_{ST}[b]	$F_{ST}max$	G'_{ST}
SEB25	0.877	0.068	0.123	0.409
SEB31	0.708	0.336	0.292	0.853
SEB33	0.918	0.032	0.082	0.694
SEB9	0.768	0.197	0.232	0.662
SAL4	0.541	0.251	0.459	0.401
SEB30	0.879	0.083	0.121	0.868
SEB37	0.888	0.051	0.112	0.580
SEB46	0.833	0.135	0.167	0.636
SAL3	0.577	0.245	0.423	0.411
SEB45	0.768	0.104	0.232	0.614
SPI10II	0.844	0.063	0.156	0.611
SPI4II	0.893	0.036	0.107	0.257
SPI6	0.792	0.079	0.208	0.396
over loci	0.791	0.126	0.209	0.541

[a] Spearman rank correlation with F_{ST} = -0.92 ($P < 0.00001$)
[b] $P < 0.00001$ for each locus and over loci

Table 3.5 Mean allospecific genetic background within each species, according to the cluster analysis performed with the program STRUCTURE. For *S. fasciatus* and *S. mentella*, values are contrasted for samples located inside and outside the Gulf of St. Lawrence–Laurentian Channel area (GSL–LCH, the area of introgressive hybridization).

Genetic background	Value in %
S. fasciatus* in *S. mentella	
inside GSL–LCH	6.4
outside GSL–LCH	2.9
areas pooled	5.8
S. mentella* in *S fasciatus	
inside GSL–LCH	2.6
outside GSL–LCH	1.2
areas pooled	1.8
S. marinus* in *S. mentella	2.0
S. mentella* in *S. marinus	1.5
S. marinus* in *S. fasciatus	1.1
S. fasciatus* in *S. marinus	4.2

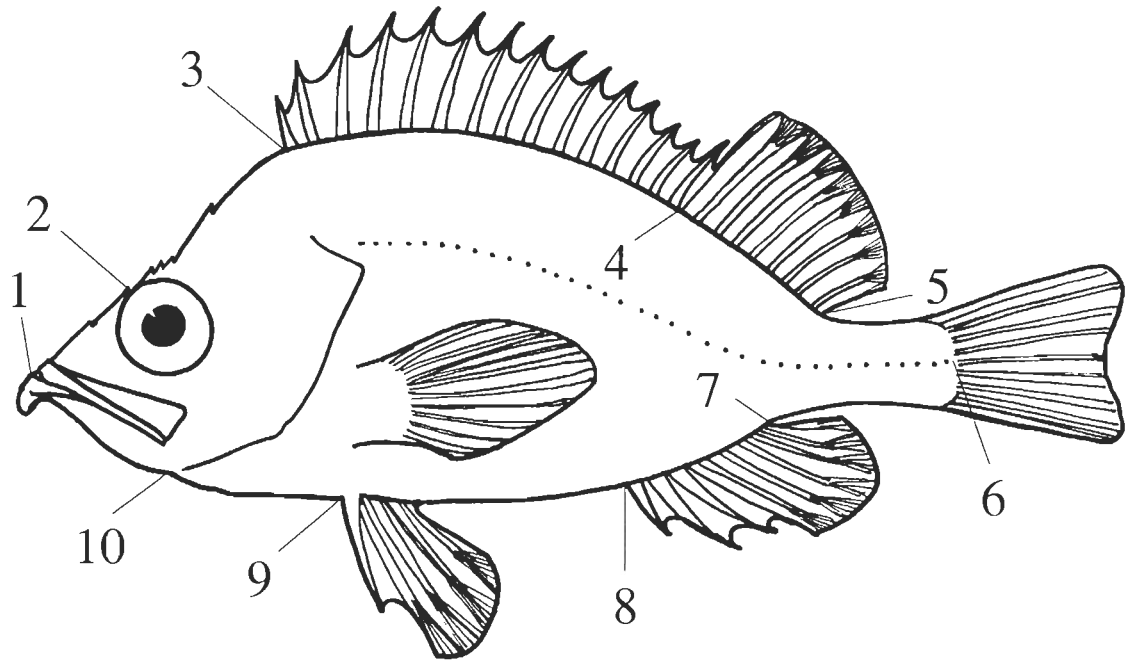

Figure 3.1 Position of the 10 landmarks used to define body shape: (1) bottom of the teeth on the lower jaw; (2) preocular spine; (3) anterior insertion of the dorsal fin; (4) posterior base of the last hard ray on the dorsal fin; (5) posterior insertion of the dorsal fin; (6) posterior extremity of the lateral line; (7) posterior insertion of the anal fin; (8) anterior insertion of the anal fin; (9) anterior insertion of the pelvic fin; (10) posterior extremity of the lower jaw (modified from Valentin *et al.*, 2002).

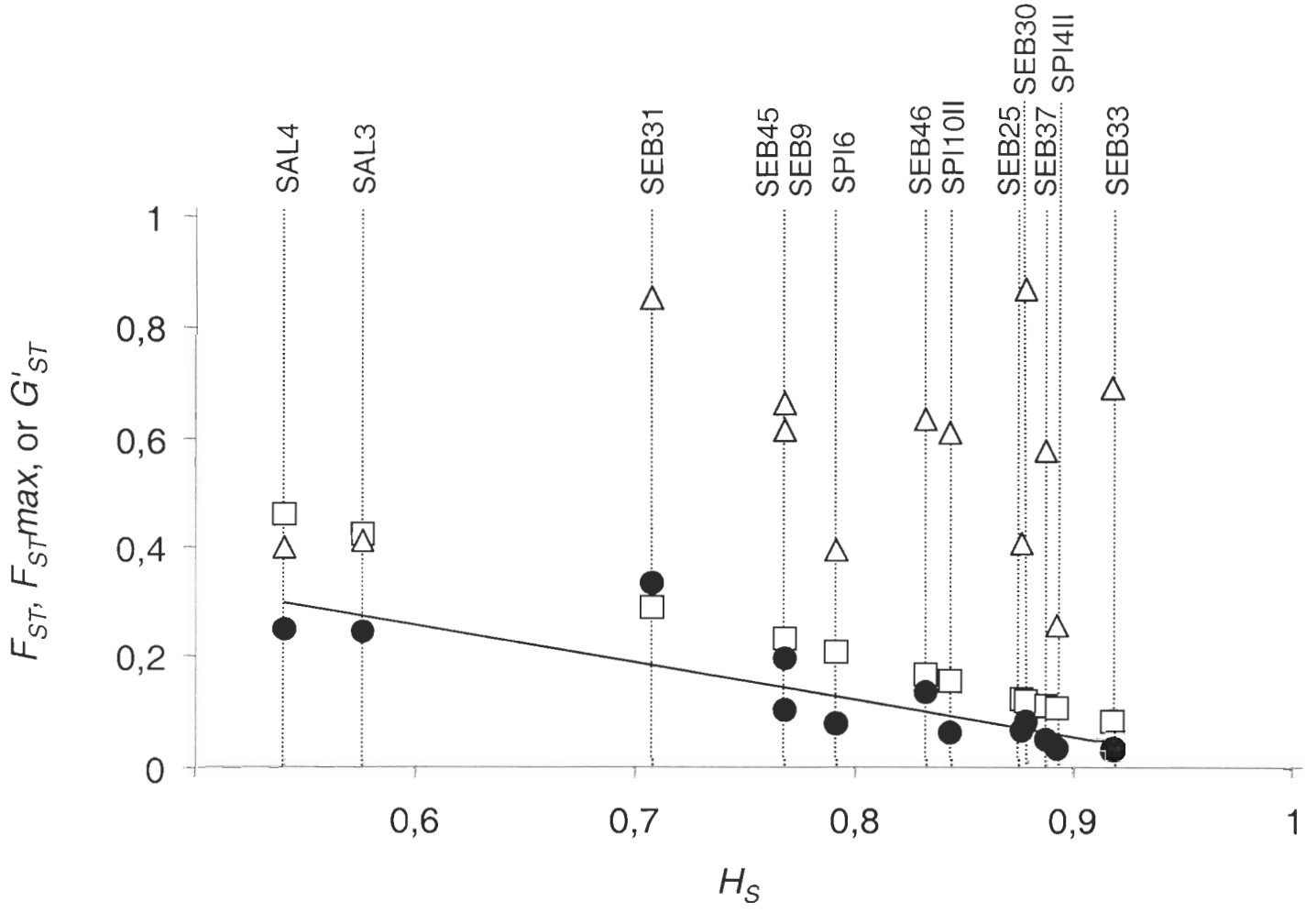

Figure 3.4 Graph showing the relationship between the locus polymorphism (represented by H_S, i.e. the mean within-sample observed heterozygosity), and the indices of differentiation F_{ST} (●), and G'_{ST} (△). $F_{ST}max$ (□) is the theoretical maximal single-locus F_{ST} value, computed as 1-H_S. The correlation (black line) between F_{ST} and H_S is significant, according to a Spearman rank test (r_S = -0.92; $P < 0.0001$).

CHAPITRE 4

COMBINING MICROSATELLITES AND GEOMETRIC MORPHOMETRICS FOR THE STUDY OF REDFISH POPULATION STRUCTURE IN THE NORTHWEST ATLANTIC

4.1 ABSTRACT

An approach combining genetics and geometric morphometrics has been used to define the population structure of two redfish species, *Sebastes mentella*, and *S. fasciatus*, in the northwest Atlantic. The genotype at 13 microsatellite loci, and body shape—defined by 10 anatomical landmarks—were determined on 1091 specimens representing 19 *S. fasciatus* and 16 *S. mentella* fishing aggregations from the northwest Atlantic. Two of the samples came from two fjords, the Saguenay and Bonne Bay. An additional *S. marinus* sample comprising 30 specimens was used for comparison purpose. A Mantel test performed on the matrices of pairwise genetic and morphometric distances between samples ($r_S = 0.71$, $P < 0.001$) revealed an overall good concordance between the two data sets. The overall genetic structure was weak within species (mean $F_{ST} = 0.010$), with only three *S. mentella* and four *S. fasciatus* samples presenting significant pairwise F_{ST} values. This weak structure was confirmed by a model-based clustering method, using a Bayesian algorithm, and by a neighbour joining tree on pairwise genetic distances between samples. Multidimensional scaling analyses (MDS) performed separately on the genetic and the morphometric data both indicated large scale geographic population structure. This structure was in agreement with the member-vagrant hypothesis. It suggested phenotypic plasticity in fish experiencing different environmental conditions and, possibly, adaptive selection of body shape. Smaller scale genetic heterogeneity was superimposed on the large scale structure. Introgressive hybridization played a role in shaping the large scale population structure, and contributed to the small scale heterogeneity, which remained in

part unexplained. The population from the Bonne Bay Fjord showed reduced allelic richness, and was genetically and morphologically differentiated. This observation supported the hypothesis of an isolated population consecutive to a founder event followed by limited gene flow. This observation was also in agreement with the hypotheses of environmentally induced phenotypic plasticity and of adaptive divergence driven by selection. The Saguenay Fjord population exhibited no loss of genetic diversity and no genetic differentiation, but was morphologically differentiated. It is proposed that the Saguenay Fjord harbours a sink population exhibiting a particular body shape as a result of environmentally induced plasticity. The implications of the observed population structure for fishery management are discussed.

Keywords: population structure, microsatellites, geometric morphometrics, redfish, fjords

4.2 INTRODUCTION

In the context of the worldwide decline of the fisheries, the identification of commercially exploited populations, or fishery stocks, has become a major issue for the implementation of sustainable fishery practices. The overall definition of stock refers to a group of randomly mating individuals maintaining a relative spatio-temporal integrity (see Carvalho & Hauser, 1994, for a review). The development of molecular techniques has encouraged the use of genetic markers to identify such reproductively isolated populations (Park & Moran, 1994; O'Connell & Wright, 1997; Ward, 2000). Genetic markers have often suggested low level of differentiation for marine species. It was interpreted as the result of large effective population sizes combined with extensive gene flow (through migration and/or larval drift) due to the open nature of the environment (Ward *et al.*, 1994; Shaklee & Bentzen, 1998; Ward, 2000).

The interpretation of population structure from genetic data is not straightforward. It relies on models, and requires acknowledging the powers and pitfalls of these models (Hellberg *et al.*, 2002, and references therein). A major concern is the question of statistically meaningful versus biologically meaningful genetic differences (Waples, 1998; Hedrick, 1999). Using highly variable genetic markers (e.g., microsatellites) has greatly raised the power of statistical tests that often failed to detect biologically significant differences with less polymorphic markers (e.g., allozymes). This high power may however result in situations were statistically significant tests do not reflect meaningful biological

differences. In the context of marine stock identification, both accuracy and precision of the statistical estimates of differentiation are particularly subject to noise, because the relative signal of differentiation is often weak. Besides, even a low migration rate (i.e., a few migrants per generation) is often extensive enough in term of gene flow to homogenize allelic frequencies, thus preventing genetic differences due to random drift, between populations (Waples, 1998). This gene flow has an important consequence for the maintenance of genetic diversity, but a very limited impact on demography. Indeed, exchanging few migrants per generation does not necessarily imply that the populations are interdependent regarding recruitment dynamics, which is of prime importance for fishery management. Such ambivalence often reflects in populations presenting low genetic differences, but significant differences for other biological characteristics such as morphology, behaviour, parasite load, and otolith elemental composition. Adopting a multidisciplinary approach combining these biological characteristics with genetic markers is therefore recommended in the context of fishery stock management (Deriso & Quinn, 1998; Grant *et al.*, 1999; Cadrin, 2000). In the nineties, the emergence of geometric morphometrics has redynamized the morphological approach for studies dealing with species and populations identification (Adams *et al.*, 2004).

Body shape is the result of a genetic program in interaction with the environment and selective forces (Alberch *et al.*, 1979; Swain & Foote, 1999, and references therein). The genetic program determines ontogenetic and physiological rates, which translate into allometric growth of body parts, which ultimately define size and shape. However, body

shape may exhibit plasticity, being modulated by environmental influences on physiological rates. Moreover, selection may act on the components of size and shape that are determinant for fertility and mortality in a given environment. Such adaptive selection favors the inheritance of particular ontogenetic and physiological rates (Alberch *et al.*, 1979; Swain & Foote, 1999, and references therein). Acknowledging the relative contribution of genetics, environment, and selection in body shape determination is important for population identification studies. This relative contribution is dependent on the species characteristics (e.g. lifespan, swimming behaviour). Long-living iteroparous species are usually characterized by small phenotypic variance, especially when environmental variations take place on short periods relative to the species life expectancy (Schultz, 1989). This characteristic is explained by selection favouring genotypes that produce a relatively narrow range of phenotypes, which are suited for the more commonly occurring class of environments. The body shape of long-living iteroparous species is expected to be under a strong genetic control, and thus to exhibit a relative low plasticity in response to the environment. Over time, selection should act on these species, favouring different mean body shapes to emerge in populations experiencing different ranges of environmental conditions.

Fjords represent natural laboratories to empirically investigate the evolutionary processes associated with the effects of isolation in marine populations. Fjords are often characterized by particular environmental conditions in comparison with the open marine waters. In many cases, gene flow is reduced between the two environments, because of

topology, and of water characteristics and dynamics. Isolated fjord populations eventually build up genetic and phenotypic differences, through genetic drift, phenotypic response to the environment, and selection (e.g., Suneetha & Naevdal, 2001; Sköld *et al.*, 2003; Jørstad *et al.*, 2004; Perrin *et al.*, 2004).

North Atlantic redfish species are long-living iteroparous species. They consist of a complex of three species currently identified as *Sebastes mentella* and *S. fasciatus*, which dominate the commercial fishery, and *S. marinus*, which occurs at much lower abundance (Ni & McKone, 1983; Atkinson, 1987; Rubec *et al.*, 1991; Gascon, 2003; Valentin *et al.*, 2006). Although recruitment of redfish is known to be sporadic in the northwest Atlantic, there has been almost no important recruitment in the last 20 years. The fishery is closed since 1995 in the Gulf of St. Lawrence (Unit 1 – Div. 4RST+3Pn4Vn [Jan-May]) and quotas have been generally declining in other areas (DFO, 2001*a*). In view of general low stock abundance, and of absence of significant recruitment, it was very important to understand redfish stock structure and their inter-relation.

The 1996-1998 Multidisciplinary Research Program on Redfish (funded by the Canadian Department of Fisheries and Oceans) gave some important initial results with respect to unravelling the complex population structure of *S. fasciatus* and *S. mentella*, and gave additional insight into the basic biology of redfish (Gascon, 2003). The systematic application of microsatellite markers has allowed the clear discrimination between *S. fasciatus* and *S. mentella* in the northwest Atlantic (Roques *et al.*, 1999a; 1999b; see also

Chapter 3). These results were corroborated by body shape analysis, with *Sebastes mentella* being more fusiform than *S. fasciatus* (Valentin *et al.*, 2002). Assuming a correlation between body shape and swimming behaviour (Blake, 1983), and that a fusiform shape is characteristic of migratory species, this study suggested a more sedentary behaviour in *S. fasciatus*. This hypothesis was consistent with the relative distribution of the two species. *Sebastes fasciatus* is distributed at shallower depth (i.e., 150-300m), and over a smaller geographical area (i.e., from the Gulf of Maine, to the Gulf of St. Lawrence, and the Newfoundland Grand Bank, reaching its northernmost limit in the Labrador Sea). In comparison, *S. mentella* is more pelagic, being distributed at deeper depth (i.e., 300-500m), and all over the North Atlantic (i.e., from the Gulf of St. Lawrence northward, including Greenland, Iceland, and Norway waters) (Atkinson, 1987; Gascon, 2003; Valentin *et al.*, 2006). Previous studies also showed that the two species undergo introgressive hybridization, essentially in the Gulf of St. Lawrence (Unit 1, hereafter called GSL), and along the Laurentian Channel south of Newfoundland (Unit 2, hereafter called LCH) (Rubec *et al.*, 1991; Desrosiers *et al.*, 1999; Roques *et al.*, 2001; Valentin *et al.*, 2006; see also Chapter 3).

As expected for marine species, the two species exhibited weak population structure (Roques *et al.*, 2001; 2002). For both species, it was suggested that introgressive hybridization played a role in shaping population structure, and contributed, along with selection, to the relative differentiation of the GSL-LCH. Additional hypotheses were presented to explain the population structure of *S. mentella*, which was more extensively

investigated. At a large geographical scale, the member-vagrant hypothesis (Sinclair, 1988, and references therein) was proposed to explain the presence of three main population groups, corresponding to the western (i.e., GSL–LCH), the central, and the eastern parts of the North Atlantic Ocean. This hypothesis assumes that the number of distinct populations within marine species is mainly determined by the number of environmental settings (oceanographic, physical) that retain young life history stages (larvae, juveniles) and favour their growth and survival. This hypothesis is criticized for disregarding density-dependant (biotic) mechanisms as a factor of population regulation (e.g., Zeldis, 1989).

Though very valuable, these first studies based on microsatellites only provided an overall description of the population structure. A finer resolution was needed for the purpose of fishery management. The objective of the present study is to further document the population structure of *S. mentella* and *S. fasciatus* in the northwest Atlantic, bringing many innovations regarding methodological, and statistical procedures, as well as sampling strategy and coverage. A new multidisciplinary approach combining geometric morphometrics with microsatellites is a first asset of the project. Assuming the role of the member-vagrant model in shaping population structure, it is predicted that body shape should vary between populations experiencing different environmental conditions, because of some phenotypic response to the environment (i.e., plasticity) or of selective adaptation. A second innovation is to target the large aggregations that are known to sustain the fishery, and to perform the sampling during the mating period, when the genetic population structure is determined. The sampling coverage is also more extensive, especially in the

GSL–LCH area, where the debate on the population structure is exacerbated by commercial considerations. In addition, the analysis of summer samples from the GSL–LCH area allows assessing the seasonal variability in the pattern of population structure. A third asset of the study is to improve the genetic approach by (i) increasing the number and the quality of microsatellite loci (i.e., adding four tetra- and one pentanucleotide microsatellite loci) and (ii) using new statistical methods, among which a Bayesian clustering approach requiring no *a priori* assignment of the specimens. Besides, for the first time, microsatellite analyses are performed on *S. fasciatus* specimens from the Grand Bank area, and from the Bonne Bay Fjord. The sample from Bonne Bay gives the opportunity to empirically investigate the effect of population isolation on gene flow and its evolutionary outcome.

It has to be mentioned, that this study provides additional insight at the species level, revealing for example a possible participation of *S. marinus* in the introgressive hybridization process. This information is presented elsewhere (see Chapter 3).

4.3 MATERIAL AND METHODS

4.3.1 Samples

A total of 3211 redfish (representing 57 sets) were collected all over northwest Atlantic Fisheries Organization (NAFO) fishing areas in summer and fall 2001 and 2002,

with a bottom-trawl net. For each set, an average of 60 fishes was frozen immediately on board ship. Redfish from the Saguenay Fjord (Sag) were caught using handlines during ice-fishing in winter 2003, while specimens from the Bonne Bay Fjord (BonBay) were caught in the East Arm, in spring 2002. Specimens were processed, selected, and assigned to their respective species as described in Chapter 3. This procedure resulted in a total of 1121 specimens, representing 36 monospecific samples – 16 *S. mentella*, 19 *S. fasciatus*, and one *S. marinus* – each comprising an average of 30 specimens (Table 4.1; Figure 4.1). For each specimen, the following data were collected – fork length, sex, maturity, overall body shape (as defined by 10 landmarks), genotype at 13 microsatellite loci, and the three traditional criteria used for species discrimination. These three criteria are the genotype at the *MDH-A** locus, the number of soft anal fin rays (AFC), and the insertion pattern of the gasbladder muscle between ribs (EGM) (Gascon, 2003; Valentin *et al.*, 2006, and references therein). The protocols used for capturing the morphometric data, and for the amplification and analysis of the 13 microsatellite loci were described elsewhere (see Chapters 2 and 3).

4.3.2 Statistics on genetic data

4.3.2.1 Descriptive statistics

The data set describing the genotype at the 13 microsatellite loci (36 samples, N_{tot} = 1121) was analyzed with MICRO-CHECKER (Van Oosterhout *et al.*, 2004) to detect scoring

incongruities and possible null alleles. The number of private alleles was determined for each sample using the software CONVERT (Glaubitz, 2004). The number of alleles, and allelic richness (A) were calculated for the 36 samples, at each locus, using the software FSTAT (Goudet, 2001). Observed (H_o) and expected (H_e) heterozygosities were calculated for each sample at each locus using the software ARLEQUIN (Schneider *et al.*, 2000). H_o and H_e over loci by sample and over samples by locus were computed using softwares GENETIX (Belkhir *et al.*, 1996–2004) and FSTAT (Goudet, 2001), respectively. Allelic richness and heterozygosity were compared between species, using permutation tests. The software FSTAT (Goudet, 2001) was also used to estimate F_{IS} (i.e., the heterozygote deficit within samples) per locus and over loci for each sample, and to estimate F_{IT} (i.e., the global deficit in heterozygote). F_{IS} and F_{IT} were tested (10 000 randomizations) for significant differences from zero to assess the compliance with the Hardy-Weinberg equilibrium. Linkage disequilibrium was tested within samples between all pairs of loci, using ARLEQUIN (50 initial conditions and 1 000 permutations; Schneider *et al.*, 2000). All probability values were adjusted for multiple comparison tests using sequential Bonferroni adjustments (Rice, 1989).

4.3.2.2 Bayesian clustering method with the program STRUCTURE

The program STRUCTURE (Pritchard *et al.*, 2000; Falush *et al.*, 2003) was used to infer population structure, along with assigning individuals to populations, and identifying

migrants and admixed individuals. This program implements a model-based clustering method. Individuals are assigned probabilistically to individual populations, or jointly to two or more populations if their genotypes indicate that they are admixed. It is assumed that the loci within populations are at Hardy-Weinberg equilibrium, and linkage equilibrium. The individuals are assigned to populations in such way as to achieve these assumptions. For each individual, the program computes the proportion of the genome originating from each population, and displays the results graphically. When unknown, the number of populations (K) is also inferred probabilistically. A first analysis was performed on the complete data set (i.e., data from the three species), as described in Chapter 3. Then, the program (with default settings) was run separately for *S. mentella* and *S. fasciatus* specimens (three simulations per K, with K ranging from 1 to 6) with a burn-in period of 500 000 steps followed by 1 000 000 Markov chain Monte Carlo (MCMC) repetitions.

A second batch of analyses was performed with the program STRUCTURE to investigate whether the observed population structure within *S. mentella* and *S. fasciatus* was driven by the presence of introgressed specimens in the GSL–LCH samples. The rational was to reanalyze the data after removing all introgressed specimens. For a given species, the first STRUCTURE analysis revealed the presence of a genetic background originating from the two alternative species (see Chapter 3). Outside the GSL–LCH area, this genetic background averaged 2.9% for *S. mentella* and 1.2% for *S. fasciatus*. These values were chosen as a threshold for discarding the introgressed specimens, keeping only

"pure" *S. mentella* (N = 124), and "pure" *S. fasciatus* (N = 265), for the second batch of STRUCTURE analyses.

4.3.2.3 Genetic differentiation: F_{ST} and G'_{ST}

For both *S. mentella* and *S. fasciatus*, genetic differentiation between pairs of samples was quantified by estimates of pairwise fixation indices (F_{ST}) based on variance in allelic frequencies according to Weir & Cockerham (1984), available in ARLEQUIN (Schneider *et al.*, 2000). The computations were based on allelic frequencies, because several loci did not follow stepwise mutational model. Test of pairwise differences for F_{ST} values were computed using 100 000 permutations with probability values adjusted for multiple comparisons, using Bonferroni sequential correction.

For both *S. mentella* and *S. fasciatus*, the amount of genetic differentiation over samples (F_{ST}) was estimated locus-by-locus, as well as over loci, using the AMOVA module available in ARLEQUIN (Schneider *et al.*, 2000). The number of permutations was set to 1 000 for statistical tests of significance. The G'_{ST} index of differentiation (standardized for the effect of polymorphism; Hedrick, 2005) was also computed.

4.3.2.4 Non-metric multidimensional scaling on genetic distances

The extent of genetic divergence among pairs of samples was also quantified by Cavalli-Sforza & Edwards (1967) chord distance (D_{CE}). The matrix of distance was submitted to a multidimensional scaling analysis or MDS (Kruskal, 1964a & b) using SYSTAT (©2002, SYSTAT Software Inc.). MDS is an iterative ordination procedure based on monotone regression. It allows representing distances between objects in a reduced space. This space has no dimension, since it is based on ranks, and it is chosen in a way to maximize the representation of true distances between objects. The level of concordance between the representation of the distances and the true distances is quantified by a measure of stress. Kruskal (1964a & b) suggested the following qualitative-quantitative evaluations for the goodness of fit associated with various levels of stress: poor (0.40), fair (0.20), good (0.10), excellent (0.05), and perfect (0.00). The MDS analysis was performed with all 36 samples from the 3 species. This was done to assess the role of introgressive hybridization in shaping population structure (see also Chapter 3). The scores of the MDS analysis were plotted. Each sample was represented by a symbol which size was proportional to the mean centroid size of the specimens. This graph allowed illustrating whether the observed pattern may represent a temporal effect, keeping in mind that the mean centroid size is only a rough approximation of the individual length composition. To illustrate the influence of introgression between *S. fasciatus* and *S. mentella* on the observed pattern, the symbol of the samples in which most introgression was observed were filled with the color of the species they were introgressed with.

4.3.2.5 Neighbour-Joining tree on genetic distances

The D_{CE} matrix of distances was also used in Neighbour-Joining tree (NJ tree) computations. The modules SEQBOOT, GENDIST, NEIGHBOUR, and CONSENS (available in package PHYLIP v.3.6, Felsenstein, 2002) were successively conducted to build the tree. Confidence estimates on tree topology were estimated by 1 000 bootstrap resampling. Performing both MDS and NJ tree on the D_{CE} matrix of distances may appear redundant, but it allowed comparing the results of the present study with those of Roques *et al.* (2001; 2002).

4.3.3 Statistics on geometric morphometric data

4.3.3.1 Artefact correction and individual pooling

Morphometric data of the 1121 specimens representing the 36 samples were submitted to a Generalized Procrustes Analysis (GPA) using software tps RELATIVE WARPS (Rohlf, 2003c). This procedure translates rotates and scales (to unit centroid size) the original configurations in order to achieve the best superimposition of all shapes. The new coordinates, called superimposed coordinates, were corrected for upward/downward arching artefact (see Chapter 2). All subsequent analyses were performed on the corrected coordinates.

Generally, large morphometric samples include several specimens belonging to the same group (e.g., the same sex, species, etc.). This redundant information is eliminated by taking the mean group configuration. Pooling specimens belonging to the same group allows to decrease the total variance and to minimize the influence of individual errors. This approach was particularly relevant to the present study, since the arching artefact revealed that individual errors were a significant source of variability (see Chapter 2). Thus, the data were pooled by species, sex and sample, and the mean configuration of each group was calculated, leading to a total of 72 groups (i.e., 1 *S. marinus* sample, 16 *S. mentella* samples, and 19 *S. fasciatus* samples for both sex).

4.3.3.2 Sample pooling

A statistical analysis was performed to test whether the morphometric data for both sexes may be pooled to address the question of population structure within *S. fasciatus* and *S. mentella*. For each species, the pairwise Euclidian distances between samples were calculated within sex, and the correlation between the two matrices was tested through a Mantel test with 1 000 permutations using software NTSYSpc (Rohlf, 2002). In parallel, for both species, each matrix of distances between samples within sex was submitted to a separate MDS (Kruskal, 1964a & b). Within each species, the MDS results for males and females were convergent in describing population structure. Besides, the matrices of distances between samples for males and females were significantly correlated (r_S = 0.87,

$P \leq 0.001$ for *S. mentella*; $r_S = 0.59$, $P \leq 0.001$ for *S. fasciatus*). Therefore, the data for males and females were pooled, leading to 36 mean configurations, representing the 36 samples (i.e., 1 *S. marinus*, 16 *S. mentella*, and 19 *S. fasciatus*).

4.3.3.3 Non-metric multidimensional scaling on morphometric distances

The pairwise Euclidean distances between the 36 mean configurations were calculated, and the distance matrix was submitted to a MDS. The scores of the MDS analysis were plotted. To illustrate the eventual influence of introgression between *S. fasciatus* and *S. mentella* on the observed pattern, the symbol of the samples in which introgression was observed (on the basis of microsatellites) was filled with the color of the species they were introgressed with. For each sample, the symbol size was also proportional to the mean centroid size of the specimens. This allowed illustrating whether allometry played a role in the observed pattern (i.e., when significant shape differences were observed between two samples whose specimens had a different mean centroid size.

4.3.3.4 Testing for allometry

Additional statistical analyses were performed on individual data, when allometry was suspected to drive the differentiation pattern. A method to disentangle shape variance

accounted for by specimen size and sample origin is easily performed by a projection of the individual data orthogonally to the common allometric vector (calculated from the mean variance-covariance matrix of the samples being compared), followed by a discriminant function analysis on the projected coordinates. The ideal condition for this procedure to apply is when the range of specimen size within sample is large enough to infer biologically significant allometric vectors within sample. When these conditions were not met, the body shape of the few specimens that shared a similar range of body size between the samples was compared through a relative warp analysis.

4.3.4 Comparing genetic and geometric morphometric data

The correlation between the matrix of D_{CE} genetic distances and the matrix of Euclidean morphometric distances between pairs of samples was tested through a Mantel test with 1 000 permutations. This test was done to assess the overall concordance between the two data sets, in describing population structure. This concordance was also evaluated in a more descriptive way, by comparing the MDS graphic representations generated by the two approaches.

4.4 RESULTS

4.4.1 Descriptive statistics

A summary of the standard descriptive statistics—number of alleles, number of private alleles, allelic richness (A), observed (H_o) and expected (H_e) heterozygosities, and tests for deviation from Hardy-Weinberg proportions (HW)—is given in Table 4.2.

The total number of alleles reached 375. Within species, 4 (3PS88b, 4VN5, s261, and s327) out of the 19 *S. fasciatus* samples, and all but one (3PS133) of the 16 *S. mentella* samples presented private alleles. All these private alleles were rare alleles that were found only once or twice overall the total data set. The number of private alleles was positively correlated with the number of alleles within loci ($P < 0.011$).

Overall genetic variability was high, with allelic richness (A) per sample averaged over loci ranging from 7.9 (BonBay) to 12.7 (2J42) (mean = 11.0). Allelic richness was significantly higher for *S. mentella* samples than for *S. fasciatus* ones ($P < 0.001$, based on 1 000 permutations), with values ranging from 11.2 (s1050) to 12.7 (2J42) (mean = 12.0), and 7.9 (BonBay) to 11.8 (3PS88b) (mean = 10.2), respectively. For the single *S. marinus* sample, allelic richness (11.5) was closer to *S. mentella* mean value. Allelic richness per locus averaged over samples varied between 5.2 (SAL3) and 21.8 (SEB30) (mean = 13.1). Allelic polymorphism was lower for SAL loci than for SEB and SPI loci.

Overall, heterozygosity was high with H_o values ranging from 0.673 (BonBay) to 0.817 (3PN77) (mean = 0.745). Heterozygosity was significantly higher for *S. mentella* samples relative to the *S. fasciatus* ones ($P < 0.001$, based on 1 000 permutations), with H_o values ranging from 0.695 (2J42) to 0.817 (3PN77) (mean = 0.765), and 0.673 (BonBay) to 0.757 (3PS1 and 3PS138) (mean = 0.727), respectively. For the *S. marinus* sample (3PN73), H_o (0.790) was closer to *S. mentella* mean value.

Significant departure (after Bonferroni sequential correction) from Hardy-Weinberg proportions (HW)—all associated with a heterozygote deficit—was observed in 2 samples belonging to *S. mentella* (2J42 and 3L29). Considering the stringency of the Bonferroni correction, it has to be mentioned that nine other samples (s1050, 4VS147, and sag for *S. mentella*; 3O44, 3PS114, 3PS26, s261, s266, and s327 for *S. fasciatus*) presented low *P* values (0.001 to 0.005; Table 4.2), indicating that departure from HW might be also present within these samples. The locus-by-locus analysis indicated that significant HW departure (after Bonferroni sequential correction) was present at loci SEB33, SEB37, and SPI10II, which were among the most polymorphic loci (Table 4.2). Locus SEB46 presented values close to significance. The HW departure involving SEB37 and SEB46 was only observed within the *S. mentella* samples. The analysis performed with MICRO-CHECKER (Van Oosterhout *et al.*, 2004) suggested the presence of null alleles at all loci exhibiting significant HW departure.

A total of 175 cases of linkage disequilibrium were observed on a possibility of 2808 (i.e., 6.2%). Significant linkage disequilibrium (after Bonferroni sequential correction) was observed in *S. mentella* for sample 4VS13 between loci SEB33 and SPI10II, and for sample 2J42 between loci SEB46 and SPI6, as well as in *S. fasciatus* for sample BonBay between loci SEB31 and SEB45, and for sample 3O44 between loci SEB30 and SPI4II.

4.4.2 Bayesian clustering method with the program STRUCTURE

The analysis performed on the total data set comprising redfish from the three species revealed eight most probable populations ($P_{K=8} \cong 1$), which in turn clustered in three well defined groups representing the three species (Figure 4.2A). Among the eight populations, one corresponded to *S. marinus*, two to *S. mentella*, and five to *S. fasciatus*. The genetic make-up of each specimen was clearly dominated by one species, but the presence of genetic background originating from the two other species was observed. These results have been presented and discussed thoroughly in another paper focusing on the issue of interspecific admixture (see Figure 3.5 and discussion in Chapter 3). For that purpose, the same color was used for all populations corresponding to the same species. All *S. mentella* samples and half of the *S. fasciatus* samples (4R107, 4VN67, 3PS88b, and 3PS114) from the GSL–CHL area showed evidence of introgressive hybridization, with several specimens presenting increased genetic background originating from the alternative

species. Three *S. fasciatus* specimens from the Gulf of Maine (s266) showed sign of introgression with either *S. mentella* or *S. marinus*. In the present study, the same results are presented (Figure 4.2A) in a way to address the issue of population structure within species, using one color for each population identified by the program.

Analyses performed separately on *S. mentella* and *S. fasciatus* samples confirmed the presence of two populations within *S. mentella* ($P_{K=2} \cong 1$; Figure 4.2B), but revealed only three populations within *S. fasciatus* ($P_{K=3} \cong 1$; Figure 4.2C). Within species, the genetic make-up of a given specimen comprised a mix of the characteristics from the different populations, with a tendency for one population to be dominant. Each sample comprised specimens showing different genetic make-up, with a tendency for one genetic make-up to be dominant. This combination of individual genetic make-up and specimen association gave a genetic signature to each sample. For *S. fasciatus* (Figure 4.2C), samples 3L65, 3N23, 3O44, 3PS138, 3PS26, and NS95 (originating from around the Grand Bank, and from the slope of the Nova Scotia shelf) exhibited a signature dominated by the "green" population. Samples 4R53, 4VN5, BonBay (from the GSL–LCH area), and NS85 (from the Nova Scotia shelf) were dominated by the "light-yellow" population, while 3PS88b, 4R107, and 4VN67 (from the GSL–LCH area) were dominated by the "blue" population. The remaining samples form the GSL–LCH area and from the Gulf of Maine exhibited intermediate signatures. For *S. mentella* (Figure 4.2B), the genetic signature of every sample was dominated by the "pink" population, except for 2J42 dominated by the red population, and for 3L29, and s1050 showing intermediate signatures.

Posterior probabilities for the STRUCTURE analysis performed on the pure *S. mentella* specimens (i.e., pure at more than 97.1%) suggested that the most probable number of population (K) was one, which would indicate no population structure in absence of the introgressed specimens. However, the differences between the northern samples (2J42, 3L29, and s1050) and those of the GSL–LCH area were detected for $K = 2$, even if this scenario was considered as improbable ($P = 8 \cdot 10^{-123}$). In the same way, the STRUCTURE analysis performed on the pure *S. fasciatus* specimens (i.e., pure at more than 98.8%) confirmed the population structure observed with the total data set for $K = 3$, even if the probability for this scenario was very low ($P = 3 \cdot 10^{-14}$) and inferior to the probability of $K = 1$ ($P = 8 \cdot 10^{-5}$) or $K = 2$ ($P = 5 \cdot 10^{-11}$).

4.4.3 Genetic differentiation

For *S. fasciatus*, pairwise F_{ST} values between samples (Table 4.3) ranged from -0.005 (s266 vs s327, two samples from the Gulf of Maine) to 0.052 (3L65 vs BonBay). All of the significant pairwise differences (after Bonferroni sequential correction) comprised the sample from Bonne Bay, samples 4R53, 4VN5 (from the GSL–LCH area), or sample NS85 (from the Nova Scotia shelf). Samples 4R53, 4VN5 and NS85 were not significantly different from each other. The sample from Bonne Bay was the only one to be significantly different from all others *S. fasciatus* sample. For the *S. mentella* samples, pairwise F_{ST} (Table 4.4) ranged from -0.003 (3PN1 vs 4S35) to 0.043 (2J42 vs 3PS133). All of the

significant pairwise differences (after Bonferroni sequential correction) comprised the northernmost samples s1050, 3L29, or 2J42, which were significantly different from all samples from the GSL–LCH area. The sample 2J42 was the most differentiated, but not significantly different from 3L29, which neither was significantly different from s1050.

For both species, most of the variation was explained by differences within samples. Though small, the percentages of variation between samples were highly significant ($P < 0.00001$), reaching 0.94% for *S. mentella*, and 1.02% for *S. fasciatus*. For *S. mentella*, the locus-by-locus AMOVA revealed seven loci with significant F_{ST} values ($P < 0.001$, after Bonferroni sequential correction) (Table 4.5). The highest F_{ST} were found at loci SAL3 (0.022), SAL4 (0.019), and SEB45 (0.018). The highest G'_{ST} values were observed at loci SEB45 (0.140) and SEB30 (0.104) (Table 4.5). For *S. fasciatus*, eight loci showed significant F_{ST} values (Table 4.5), which were the highest at loci SEB33 (0.015), SEB30 (0.014), and SEB45 (0.014). Locus SEB31 presented the highest F_{ST} value (0.019), but also the highest error for this estimate (0.012), which could explain why it was not significant. The highest G'_{ST} values were observed at the most polymorphic loci, SEB33 (0.131), SEB30 (0.121), SEB37 (0.106), SPI4II (0.091), and SPI10II (0.083) (Table 4.5).

4.4.4 MDS on genetic distances

MDS graphic representation of genetic distances between the 36 samples was good with a stress value reaching 0.031 (Figure 4.3). The three species were clearly differentiated. For *S. mentella*, the three northern samples (2J42, 3L29, and s1050) were apart from the GSL–LCH ones, which formed a homogeneous cluster. These *S. mentella* GSL–LCH samples were logically closer to the *S. fasciatus* samples, because of introgressive hybridization. For *S. fasciatus*, the samples from the GSL–LCH area were dispersed. The samples exhibiting low levels of introgression (4VS36, 4R53, 4VN5) were close to the samples from the Gulf of Maine and from the Nova Scotia shelf (s261, s266, s327, and NS85), while the samples exhibiting higher level of introgressive hybridization (4R107, 4VN67, 3PS88b, and 3PS114) were pulled towards *S. mentella* and *S. marinus*. The *S. fasciatus* samples from around the Grand Bank and from the slope of the Nova Scotia shelf (3L65, 3N23, 3O44, 3PS138, 3PS26, and NS95) were close together. The Bonne Bay sample was differentiated, although closer to the samples with low introgression levels from the GSL–LCH, the Gulf of Maine, and the Nova Scotia shelf. No particular grouping related to the mean centroid size of the specimens was observed, suggesting that the pattern was not driven by a temporal effect.

4.4.5 NJ tree on genetic distances

The NJ tree based on D_{CE} genetic distances between the 36 samples representing the three species clearly illustrated the genetic separation between the three species with bootstrap values of 100% (Figure 4.4). Overall, *S. mentella* showed less structure than *S. fasciatus*. For *S. mentella*, all samples from the GSL–LCH area clustered together on the tree, but were different from the northernmost samples (2J42, 3L29, and s1050) with an 88% bootstrap value. Among the northern samples, s1050 and 3L29 clustered together, leaving out 2J42 in 74% of the cases. For *S. fasciatus*, a first branch (bootstrap = 70%) regrouped samples from all around the Grand Bank (3L65, 3N23, 3O44, 3PS1, 3PS26, and 3PS138) and from the slope of Nova Scotia shelf (NS95). Four samples from the GSL–LCH area (4R107, 4VN67, 3PS88b, and 3PS114) were located on the second branch that was connected with *S. marinus* and *S. mentella*. The extremity of the third branch (bootstrap = 91%) encompassed four samples from different geographic origin—NS85 from the Nova Scotia shelf, 4R53 from the GSL, 4VN5 from the LCH, and the sample from Bonne Bay. The samples from the Gulf of Maine (s261, s266, s327) were located at the basis of the third branch.

4.4.6 Geometric morphometrics

MDS graphic representation of morphometric distances between the 36 samples was good with a stress value reaching 0.088 (Figure 4.5). The only *S. marinus* sample (3PN73) was closer to *S. fasciatus* from southern Grand Bank (3O44). The samples of *S. fasciatus* and *S. mentella* showing sign of introgression (according to microsatellite data) were not closer than samples of the two species encompassing "pure" specimens. In fact, the smallest morphometric distance between the two species was between the *S. mentella* sample from Labrador (2J42), and the *S. fasciatus* sample from northern Grand Bank (3L65). The *S. mentella* samples from the GSL–LCH area, which all comprised introgressed specimens (according to microsatellites), formed a homogeneous group, suggesting that introgression or geography was involved in the observed pattern. However, the sample from the Saguenay Fjord (Sag) made exception and was differentiated from the group. The northern *S. mentella* samples were different from the GSL–LCH ones, with the sample from Greenland (s1050) being further apart from the Labrador Sea (2J42) and the northern Grand Bank (3L29) samples. The northern (2J42, 3L29, and s1050) and the Saguenay (Sag) samples were composed of specimens of smaller centroid size compared with the group of samples from the GSL–LCH area, suggesting that allometry may play a role in the observed pattern.

The overall distribution of *S. fasciatus* samples on the MDS graph was more spread and less structured than for *S. mentella.* However, samples tended to be distributed

according to their geographical origin. The samples from the Grand Bank area (3L65, 3N23, and 3O44), from the southern tip of St. Pierre Bank (3PS1, 3PS138, and 3PS 26) and from the Nova Scotia slope (NS95) were close together. The samples from the GSL–LCH area were grouped together, whether they encompassed introgressed specimens or not. The sample from Bonne Bay was more differentiated. For the samples from the Gulf of Maine, one (s266) laid with the GSL–LCH samples, one (s261) tended to be differentiated, and the last one (s327) was clearly apart. The samples with comparable mean centroid size of their specimens were dispersed, suggesting that the overall pattern was not driven by allometry. The correlation between the matrices of pairwise genetic and morphometric distances between samples was highly significant (r_S = 0.71, $P < 0.001$). This correlation was reflected by the overall convergence of the MDS analyses performed separately on morphometric (Figure 4.3) and genetic data (Figure 4.5). The matrix correlation was lower within species, but was still significant (r_S = 0.45, $P < 0.05$ for *S. mentella*; r_S = 0.43, $P < 0.001$ for *S. fasciatus*).

4.5 DISCUSSION

4.5.1 Null alleles

In the absence of null alleles, heterozygote deficit is usually thought to reflect a Wahlund effect arising from population subdivision, but assortative mating, inbreeding, and

selection may also be involved (see Roques *et al.,* 2001, and Schmidt, 2005, for a discussion on redfish). In the present study, null alleles are likely to have contributed to the significant heterozygote deficiencies observed at loci SEB33, SEB37, and SPI10II, because the homozygote excess tended to be homogeneously distributed across all homozygote classes of genotypes (Van Oosterhout *et al.*, 2004). The occurrence of null alleles at loci SEB33, and SEB37, but also at loci SEB30, and SEB46, has already been reported in *S. mentella* (Roques *et al.*, 2002; Schmidt, 2005).

In the present study, SEB33, SEB37, and SPI10II, which yielded the longest PCR products, were the most problematic loci to amplify using the routine multiplex procedure. They often demanded additional single-locus amplification to validate the results from the multiplex reaction. These technical considerations have not contributed significantly to the observation of null alleles, because large allele drop-out was not observed. Moreover, if critical, these technical problems would have caused null alleles to be observed in all samples, which was not the case.

A factor that may have contributed to the observation of null alleles is the presence of mutant primer sites. Although sequencing would be required to bring definitive conclusions, the results for the locus SEB37 supports this hypothesis. Indeed, null alleles were only detected in *S. mentella* at this locus, suggesting the occurrence of species-specific mutation(s) at the primer site(s) (O'Connell & Wright, 1997).

It is recognized that the presence of null alleles may affect all downstream statistics, and therefore alter the conclusions inferred from the results. In the present study, additional STRUCTURE and NJ tree analyses were performed without the incriminated loci. The results (not shown) were convergent with the analyses based on the 13 loci, in describing the population structure, which suggested a limited impact of the presence of null alleles.

4.5.2 Private alleles

Most private alleles were present at very low frequencies within populations. This is not surprising considering that, even at the species level, all but two private alleles were found at very low frequencies (see Chapter 3). The presence of private alleles at low frequencies is often explained by a sample size that does not cover all possible allelic states (O'Connell & Wright, 1997). This might be the case here, as suggested by the positive correlation observed between the number of alleles and the number of private alleles within loci. The presence of a low frequency private allele is not very informative regarding population structure. However, the observation of up to 5 low frequency private alleles in *S. mentella* samples 2J42 and 3L29 suggested some differentiation, which was supported by F_{ST} (Table 4.4) and genetic distances (Figure 4.3). For *S. fasciatus*, half of the private alleles were observed in samples from the Gulf of Maine (s261, s327), which would suggest some differentiation in that area, too. The observation of 4 private alleles in sample

3PS88b from southern Newfoundland is more difficult to explain, because this region is geographically less favourable to population isolation.

4.5.3 Population structure in *S. mentella*

A strong genetic homogeneity was observed for *S. mentella* within the GSL–LCH area, with none of the statistical approaches detecting significant differences between samples. This is contrasting with a previous study that reported genetic heterogeneity between samples from the area south of Newfoundland (Roques *et al.*, 2001). This heterogeneity was caused by two out of six samples and was explained as the consequence of variable levels of introgression. In the present study, different levels of introgression in the samples (Figure 3.5) did not translate into significant pairwise F_{ST} values after sequential Bonferroni correction (Table 4.4). Some lack of statistical power could not be responsible for the absence of significant differences in the present study. Indeed, the use of 13 loci instead of eight, and the absence of selection of the specimens (on the basis of the convergence between the usual criteria MDH, AFC, EGM) should in fact have accounted for more power. Besides, the observed homogeneity was remarkably confirmed by the morphometric data (Figure 4.5). Moreover, the two methods indicated that the pattern was consistent throughout the year, keeping in mind that slight body shape differences were observed between summer and fall samples across the area (not shown). Considering the good geographical coverage achieved by the samples of the two studies (this study, and

Roques *et al.*, 2001), homogeneity seems to be the rule for *S. mentella* in the GSL–LCH area. It suggests the presence of a single population for this species, in this area. The single-population hypothesis is not in agreement with the actual management practices that consider two different stocks whose limit varies seasonally (Unit1 and Unit2) (Atkinson & Power, 1991; DFO, 2001*a*).

The genetic data showed that the *S. mentella* samples from the GSL–LCH area differed from the northern samples (2J42, 3L29, and s1050). The difference between the two regions was observed with both F_{ST}-based statistics and distance-based statistics (MDS, NJ tree) computed between samples, and even with the cluster analysis (STRUCTURE) performed on specimens without prior assignment to a particular sample. This difference was further confirmed by the morphometric data, which clearly indicated that the individuals from the two areas exhibited different body shapes. A complementary analysis (where the common allometric shape changes were removed by orthogonal projection, before computing discrimination) ensured that the differences were unrelated to size (not shown). It was also empirically observed that the extrinsic gasbladder muscle was thicker in individuals from the northern samples in comparison with those of the GSL–LCH samples (personal observation). These convergent results give a strong support to previous findings indicating that *S. mentella* from the GSL–LCH area forms a population that is different from *S. mentella* inhabiting the northernmost area of the northwest Atlantic (i.e., up north from the Grand Bank). Roques *et al.* (2001; 2002) have hypothezised that the

observed pattern was related to introgression with *S. fasciatus*, and to the oceanographic characteristics of the GSL–LCH (member-vagrant hypothesis, see below).

The present study confirmed the role of introgressive hybridization in shaping population structure between the GSL–LCH and the northern area, showing that the area of introgressive hybridization was restricted to the GSL–LCH region, even if the two species shared a larger geographical zone (see Chapter 3). The locus-by-locus AMOVA between samples within *S. mentella* suggested however, that the presence of introgressed specimens in the GSL–LCH region was not the only factor responsible for the observed differentiation. Indeed, if introgression was the only factor, it would be expected that the loci implicated in the population differentiation were the same as those responsible for species discrimination. This is only partially true here, as suggested by both the F_{ST} and the G'_{ST} values (Table 3.4 in Chapter 3; Table 4.5). The analyses performed after removing the introgressed specimens from the data bring further indication that introgression does not play an exclusive role in the differentiation between the northern and the GSL–LCH areas. Indeed, the second STRUCTURE analysis on the "pure" specimens was still able to detect the two populations. Besides, the two populations were significantly different according to the F_{ST} value (0.017, $P \leq 0.00001$) calculated on pooled data (i.e. GSL–LCH samples, vs 2J42-3L39-s1050 samples). In fact, it was expected to observe the differentiation using only the "pure" specimens. Indeed, allelic richness was higher in the northern region, so that several alleles were only found in specimens from that area.

Following the member-vagrant hypothesis (Sinclair, 1988), Roques *et al.* (2001) proposed that the genetic differentiation of the GSL–LCH from the Panoceanic populations (i.e, the Grand Banks up to the Faroe Islands) was explained by the particular oceanographic conditions of the GSL, which offer a suitable environment for all life history stages, especially for the young ages. The member-vagrant hypothesis would also be consistent with the observation of morphological differences between samples from the GSL–LCH area and those from the northern regions, assuming the impact of environmental conditions on shape (through phenotypic plasticity or adaptive selection). Altogether, these results indicate that the Strait of Belle-Isle is not a route for gene flow. Unfortunately, the present study does not allow to make inference about the status of *S. mentella* inhabiting the slope of the Grand Bank in area 3N and 3O. All samples were dominated by *S. fasciatus* in that area, which is in line with the results of fishery surveys (DFO, 2001*a*). This observation itself is informative, because it suggests that the distribution of *S. mentella* is discontinuous between the northern regions and the GSL–LCH area.

The northwest Atlantic is a more open environment in comparison with the GSL–LCH area. The pattern of water circulation offers the possibility for larvae to drift from the Greenland waters to the Canadian waters, and the geographical continuity results in few barriers to adult migration. Larval and juvenile migration is thought to occur from western Greenland (which comprises redfish originating from the Irminger Sea) towards the northern Labrador Sea (Saborido-Rey *et al.*, 2001), though it has been hypothesized that the reproductive ability of these specimens was low (Troyanovsky, 1992). An international

trawl-acoustic survey showed a continuous distribution of adult redfish from the Irminger Sea to the Labrador Canadian waters (Anonymous, 2004). Former studies suggested that gene flow was high enough to prevent genetic differentiation over the Labrador, Greenland, and the Grand Bank areas (Roques *et al.*, 2002; Schmidt, 2005). Following the member-vagrant hypothesis, Roques *et al.* (2002) postulated the presence of a single and large larval retention zone, at the scale of the central North Atlantic basin, to explain the low differentiation within the panoceanic population.

The present study gave contrasting results. The three northern samples exhibited HW disequilibrium, suggesting a possible mixed origin of these samples (but see the discussion about null alleles). Besides, the sample from the Labrador Sea (2J42) exhibited highly significant genetic differences from the two other northern samples (3L29, and s1050), which were less differentiated from each other (although F_{ST} value was close to significance). These results suggest that the populations from Greenland and from northern Grand Banks are more connected together than they are with the Labrador population. Keeping in mind the results of Roques *et al.* (2001) and Schmidt *et al.* (2005), it can be hypothesized that sample 2J42 was not representative of the Labrador population. Individuals from sample 2J42 exhibited higher levels of genetic background originating from *S. marinus* and *S. fasciatus* than individuals from Greenland (s1050), and from northern Grand Bank (3L29) (see Chapter 3). This observation indicates that sample 2J42 may comprise introgressed specimens, which could explain the differentiation of this sample. It has to be mentioned that high prevalence of *Sphyrion lumpi* infestation, and of

skin bacterial infection, has been recurrently reported since the 1980s in Div. 2J (DFO, 2001*b*). This observation could be an indication of population isolation in the area. Further information is needed to infer the level of connectivity between the populations from Greenland waters (1F), the northern Grand Bank (3L), and the Labrador Sea (SA2+3K), and to determine whether the 2J42 sample represents a local population.

The morphometric data showed that the specimens from the Labrador (2J42) and from the Grand Bank (3L29) were more closely related to each other, but different from the Greenland sample (s1050). These results do not support both the strong genetic differentiation of sample 2J42, and the genetic similitude between samples 3L29 and s1050. These observations suggest that sharing the same environment promotes the morphological convergence of the two genetic groups (2J42, and 3L29). It also suggests that redfish from the Canadian waters and those from the Greenland waters represent separated populations, though a significant amount of gene flow is occurring between Greenland and the Grand Bank. However, it has to be mentioned that the Greenland sample (s1050) comprised redfish of size ranging from 22cm to 26cm, while the Grand Bank (3L29) and Labrador (2J42) samples comprised older specimens of length between 25 and 33cm. The data did not allow performing a complementary analysis investigating allometry. Therefore, it can not be ruled out that allometry is involved in the body shape differences observed between redfish from the Canadian and from Greenland waters. Additional sampling of redfish from the same size range is thus required. Such samples should not be difficult to collect considering that both areas exhibited analogous indices of

abundance at length, at least in 2000 (DFO, 2001*b*; Anonymous, 2004). This trend still needs to be validated by extensive analysis of fishery data, but it would itself suggests connectivity between the Canadian and Greenland waters, unless large-scale favourable conditions are responsible for the synchrony of recruitment pulses in different populations over a large zone.

4.5.4 Population structure in *S. fasciatus*

Sebastes fasciatus is more restricted geographically, associated with shallower waters, and characterized by a less fusiform body shape than *S. mentella* (Valentin *et al.*, 2002; Valentin *et al.*, 2006). Therefore, it is predicted for *S. fasciatus* to be more sedentary than *S. mentella*, and hence to be characterized by a stronger population structure. Overall, the results are in agreement with this prediction, suggesting local heterogeneity superimposed with larger scale trends. However, the interpretation of the results is complicated by the presence of an overall weak structure, and of contradiction between the genetic and morphometric signals of population structure.

For the first time, microsatellites have been analyzed on *S. fasciatus* specimens from around the Grand Bank (Div. 3LNO). Despite non significant F_{ST} values, the STRUCTURE, MDS and NJ analyses suggested that these specimens were part of a differentiated population (Figures 4.2; 4.3; 4.4). This population comprises the samples distributed from

along the slope of the Grand Bank (samples 3L65, 3N23, and 3O44) to the southern tip of St. Pierre Bank (samples 3PS1, 3PS26, and 3PS138), with a possible ramification on the slope of the Nova Scotia shelf (sample NS95), but no incursion into the Laurentian channel (Figures 4.3; 4.4). This pattern was supported by the MDS analysis performed on the morphometric distances, where all these samples grouped together (Figure 4.5). This grouping was not related to size, since the group was composed of samples with different mean specimen sizes. Hereafter, this population is called the northern *S. fasciatus* group.

It is interesting to mention that the northern *S. fasciatus* group showed very limited introgressive hybridization with *S. mentella*, despite the overlapping distribution of the two species in that area (see Chapter 3). In contrast, introgressive hybridization was observed in four samples from the GSL–LCH area (3PS88b, 3PS114, 4VN67, and 4R107). This observation is similar to the results obtained for *S. mentella*, and further underscores the role of introgressive hybridization in shaping population structure. Besides, even the samples from the GSL–LCH area with few introgressed specimens (4R53, 4VN5 and, to a much lesser extent, 4VS36) were differentiated from the northern *S. fasciatus* samples, indicating that additional factors are involved in the process.

For *S. mentella*, the genetic and morphometric differences between the GSL–LCH area and the northern region were consistent with the member-vagrant hypothesis. Considering that *S. fasciatus* and *S. mentella* from the GSL–LCH share overall the same environment throughout their life, it would be expected to find a homogeneous population

of *S. fasciatus* inside this area, (assuming that life-history traits are not too different between the species). This is observed for the morphometric data, with all samples from the GSL–LCH area grouping together, without distinction regarding the rate of genetic introgression (Figure 4.5). This observation is consistent with the hypothesis of phenotypic plasticity or of adaptive selection in a given environment (with some reserve, considering that samples s266 and NS85 also grouped with the GSL–LCH samples). In contrast, local genetic heterogeneity was observed in the GSL–LCH, with samples 4R53 and 4VN5 being differentiated from the other GSL–LCH samples (Figure 4.4). This heterogeneity is unlikely to be caused by different level of introgression within samples, because it was still observed with the STRUCTURE analysis performed without the introgressed specimens (not shown). Although the STRUCTURE analysis (Figure 4.2C) would suggest some affinity between 4R53, 4VN5, and BonBay, the highly significant F_{ST} values and morphometry indicate that this hypothesis is unlikely. The heterogeneity is also unlikely the result of a temporal or cohort effect, since it was not associated with specimens of a particular size. These two samples are genetically closer to the southern samples from the Gulf of Maine (s261, s266, s327) and the Nova Scotia shelf (NS85) (Figure 4.3; 4.4), but it is unlikely that samples 4R53 and 4VN5 represent immigrating populations. The southern samples showed overall concordance between the three usual classification criteria (EGM, AFC, and MDH), while samples 4R53 and 4VN5 showed a more non-concordant pattern, as characteristic of the GSL–LCH area. Altogether, these results suggest that 4R53 and 4VN5 represent local populations among the main GSL–LCH population. The existence and the persistence of

such local populations are difficult to explain, considering the life-cycle of the species and the geography/hydrography of the area.

The southern samples from the Gulf of Maine (s261, s266, and s327) and from the Nova Scotia shelf (NS85) were relatively homogeneous according to their pairwise F_{ST} values, but NS85 was differentiated according to the STRUCTURE analysis (Figures 4.2A; 4.2C), suggesting structure in the southern area. This is congruent with the results of Roques *et al.* (2001) who reported significant differences in allelic frequencies between the Gulf of Maine and the Nova Scotia shelf, in absence of significant F_{ST} values. At a larger scale, the southern samples tended to be genetically different (though weakly) from the GSL–LCH and the northern samples (Figures 4.3; 4.4), suggesting some restriction to gene flow between the areas. This is not unexpected considering that, like the Gulf of St. Lawrence, the Gulf of Maine is known as a productive environment (Conkling, 1995), where redfish are releasing larvae, and where specimens are likely to complete their life cycle (Pikanowski *et al.*, 1999; Sévigny *et al.*, 2000). The water in the Gulf of Maine is a mixture of Nova Scotia slope water (SLW), entering through the Northeast Channel, and of Nova Scotia shelf water (SHW) (Brown & Beardsley, 1978). Redfish from the Gulf of Maine are characterized by a concordant EGM, AFC and MDH pattern (Valentin *et al.*, 2006), and exhibit no sign of introgression with *S. mentella*. These observations suggest no passive larval drift from the GSL–LCH area (where introgressive hybridization is observed) through the SHW route, and no migration of older fish along the SLW route. In the mid-nineties, the redfish biomass started to increase in the Gulf of Maine. It was mostly

associated with the presence of small immature redfish produced locally during the early nineties. More recently, a relatively abrupt increase in abundance of redfish of many ages was observed in the Gulf of Maine, suggesting immigration from other areas (Northeast Fisheries Science Center, 2001). It was hypothesized that such large-scale movement among areas was related to particular environmental conditions, namely, the incursion of slope water originating from the Labrador Sea, a phenomenon that was last observed in the sixties (Clark & O'Boyle, 2001). The hypothesis of episodic pulse of immigration along the SLW route is consistent with the relative weak isolation of the Gulf of Maine. It would also explain the singularity of sample s266 from the Gulf of Maine. Unlike other southern samples, sample s266 exhibited a body shape that was more related to the GSL–LCH group. It also showed sign of introgression, and comprised heterozygote specimens at the *MDH-A** locus, and specimens with a bifid EGM. Since *S. mentella* is not supposed to inhabit the Gulf of Maine, this strongly suggests that s266 originated from outside the Gulf of Maine.

Considering the distribution and the overall population structure of *S. fasciatus*, it can be hypothesized that the populations of the GSL–LCH and the Grand Bank radiated from the southern population. This would explain the central position of the Gulf of Maine samples on the NJ tree (Figure 4.4) and on the MDS graph (Figure 4.5). This hypothesis would also be consistent with the overall concordant pattern of the three usual criteria observed in the Gulf of Maine and on Grand Bank, and with the non-concordant pattern

found in the GSL–LCH, where introgressive hybridization takes place (Rubec *et al.*, 1991; Gascon, 2003; Valentin *et al.*, 2006; this study).

4.5.5 Lessons from the fjords

The fjords of Atlantic Canada have become potential habitats for redfish following the last deglaciation that started 13 000 years ago. At that time, landmasses, including Newfoundland, were covered with ice, and sea water was above the present level. When the ice retreated, the fjords were invaded by marine waters, but were also probably under a strong freshwater influence from melting glaciers. The deglaciation was accompanied by changes of the sea level, which reached the modern situation about 6 000 years ago. High sea-level first persisted in the Saguenay region, before gradually decreasing to the present level. In contrast, about 8 000 years before present, the sea level at the mouth of Bonne Bay may have fallen by about 10 m under present level, making the sill very shallow, especially at low tide (Shaw *et al.*, 2002).

4.5.5.1 S. mentella in the Saguenay

The genetic results showed that redfish from the Saguenay Fjord (sag) belonged to the GSL–LCH population, since no significant differences were found between sag and the

samples of the GSL–LCH. On the opposite, the morphometric data indicated that the Saguenay had a characteristic body shape, which suggested an isolated population. It is unlikely that seasonal body shape variability was responsible for the observed differentiation, though the Saguenay specimens were caught in winter, and the GSL–LCH area was sampled in summer and late fall. The seasonal change of body shape is probably well below the observed differences (as suggested by seasonal body shape differences among the GSL–LCH samples). It has to be mentioned that the Saguenay sample comprised specimens of smaller size than the GSL–LCH samples. One could therefore be tempted to explain the observed differences by allometry (i.e., shape differences related to size). This is all the more tempting, considering that the three other samples (s1050, 2J42, and 3L29) that comprised smaller specimens were morphometrically closer to sag (Figure 4.5), although they were genetically equally differentiated from sag and from the GSL–LCH samples (Figure 4.3). However, the comparison of the few specimens from the Saguenay and from the GSL–LCH that shared a similar range of body size using a relative warp analysis confirmed the occurrence of a different body shape for the Saguenay specimens (not shown).

Explanations other than seasonal or allometric variations are thus needed to account for the observed morphological differences of the Saguenay specimens relative to those of the GSL–LCH area, in spite of genetic homogeneity between them. A first hypothesis would be that there is a separate population in the Saguenay, which reproduces locally. It would imply that gene flow is high enough to prevent loss of allelic richness and population

differentiation through genetic drift. The local body shape would reflect plasticity or adaptive selection, both promoted by the particular environmental conditions. A second hypothesis would be that the Saguenay specimens are mostly immigrants from the GSL, and represent a sink population. Regular migrating events would maintain the genetic homogeneity and allelic diversity of the local population. The local shape would be the result of plasticity induced by the particular environmental conditions of the fjord. Whether they were produced locally or are immigrants, it has been confirmed that the specimens from the Saguenay sample did not grow in the same environment as the specimens from the GSL. This information comes from the analyses of the otolith elemental composition (Campana *et al.*, submitted), which is known as a proxy for the environment. Regarding the possibility of local reproduction, it is known that redfish from the Saguenay Fjord show signs of reproductive activity (Jacques Gagné, pers. com., this study). However, very few redfish larvae are observed during plankton survey, suggesting a massive larval mortality in the fjord, the cause of which is still unknown (Jacques Gagné, pers. com.). Considering the hypothesis of a locally reproducing population, the question are (1) whether the weak recruitment is strong enough to sustain the observed population, and (2) whether the balance between migration and drift may be maintained under such recruitment dynamics. The alternative hypothesis of an immigrating population is more likely. It would imply that the specimens are entering the fjord after the larval stage, but mostly at a young age, to spend their life time in the area.

4.5.5.2 S. fasciatus in Bonne Bay

The results clearly indicated a strong genetic and morphometric differentiation of the Bonne Bay sample, which indicates the presence of an isolated population. Considering the history of sea-level changes in that region, it can be assumed that the initial colonization event has been followed by a period where the population became almost totally isolated, before reaching the present days conditions about 6 000 years ago. Assuming that, in absence of barriers to gene flow, 6 000 years should be sufficient to homogenize the allelic frequencies between the GSL and Bonne Bay, the actual genetic divergence is unlikely to reflect an historical artefact. A more likely explanation is that the Bonne Bay population is the consequence of a founder event, which was maintained by a limited gene flow between Bonne Bay and the GSL. The observation of lower allelic richness in the Bonne Bay sample, relative to the other *S. fasciatus* samples, is consistent with the hypothesis of a founder event. Founder event and subsequent limited gene flow has already been proposed to account for the differentiation of a population of copper rockfish (*S. caurinus*) in Puget Sound, a fjord of postglacial origin in Washington State (USA) (Buonaccorsi *et al.*, 2002).

The distinct body shape exhibited by the Bonne Bay specimens may reflect phenotypic plasticity to the particular environmental conditions of the fjord, but also adaptive selection. The body shape was so distinct that the differences were perceptible visually. A significant feature was the highest body depth, which is characteristic of a more sedentary behaviour (Webb, 1988). Another noticeable feature was the overall dark

coloration of the skin, which could also be an indication of population isolation. Coloration is assumed to reflect the diet. Recent observations on cod suggested that dark cods represented a local population, feeding and remaining in Bonne Bay year round (Gosse, 2002). Another indication of population isolation was the systematic observation of internal parasites in the Bonne Bay specimens of the present study. Altogether, the results indicate the presence of an isolated redfish population in East Arm Bonne Bay, which is likely to be self-recruiting. Considering the small size of the fjord, the population of Bonne Bay is expected to be small. It is therefore vulnerable to fishing pressure, because the local population has low potential to be reseeded from other areas. A rapid comparison between the Hardy-Weinberg equilibrium heterozygosity (H_e) and the heterozygosity expected from the number of sampled alleles (H_{eq}) suggested no recent reduction in the population size (computed with the program BOTTLENECK, version 1.2.02, Piry *et al.* 1999). Further investigation is needed to assess the consequences of fishing pressure on the sustainability and the genetic diversity of the Bonne Bay population.

4.5.6 Implications for fishery management

For *S. mentella*, managing Unit 1 and Unit 2 separately is not consistent with the observed genetic and morphometric homogeneity. Besides, depleted fishery stocks from Unit 1 and Unit 2 are unlikely to be rebuilt by specimens from the northern areas (i.e., northern Grand Bank, Labrador Sea, Greenland), and reciprocally. The low abundance of

S. mentella on the southern Grand Bank (Div. 3N+3O) has still to be validated, but suggests that this area is of little importance for *S. mentella*. In the northern area, the presence of significant gene flow is confirmed between the Canadian and the European fishery stocks, but morphometry suggested that these stocks could not be considered as a single population (with some reserves). The presence of local population(s) in the Labrador Sea has still to be validated, but could be of limited importance for the commercial fishery, because of the slow reproduction rate and the overall low abundance observed in that area.

Presently, Div. 3L+3N are managed as a single stock, Div. 3O is managed separately, and south of 3PS belongs to Unit 2. For *S. fasciatus*, genetics and morphometrics suggest that redfish in Div. 3LN+3O+south of 3PS belongs to one population. Analyses of demographic data should be performed in order to confirm that this "around Grand Banks" population is self-sustaining and could be managed as one. However, it has to be considered that sample 3L65 was systematically located at the extremity of the cluster comprising the "around Grand Banks" samples (Figures 4.3;4.4; 4.5), and that Div. 3O and south of 3PS are more prone to be influenced by migration events originating from or towards the Laurentian Channel. So, merging Div. 3O and south of 3PS, but keeping them separated from Div. 3LN, could be in line with a precautionary approach, with Div. 3O and south of 3PS acting as a buffer zone between the Laurentian Channel and the northern Grand Banks. The status of *S. fasciatus* inhabiting the southern Labrador Sea has still to be investigated. The differences observed between the GSL–LCH area and the southern regions (i.e., Nova Scotia shelf and Gulf of Maine) are consistent

with separate management practices, but support the hypothesis of episodic pulses of migration between the areas. It has still to be investigated whether such pulses would be sufficient to significantly contribute to the fishery. Managing Unit 1 and Unit 2 separately is not biologically consistent with the morphometric homogeneity observed between samples from the GSL–LCH area. However, managing this area as a single population would not consider the genetic heterogeneity observed within this area. Understanding the origin of this heterogeneity and the relative contribution of the different subpopulations to the fishery is primordial for sound management.

Considering the morphological similarity of the two species, it would be difficult to manage these species separately in Units 1 and 2. The definitive strategy should maybe consider the relative contribution of the species to the fishery to define management priorities. However, even this issue is not straightforward. Historically, in Unit 1 and Unit 2, the major pulses of recruitment were dominated by *S. mentella*. So, prioritizing *S. mentella* (i.e., by managing Units 1 and 2 as a single unit) would be important to allow this species to rebuild stocks. However, the last two documented pulses of recruitment in Units 1 and 2 were associated with *S. fasciatus* (cohorts of 1985, and of 1988). Although these cohorts disappeared before contributing significantly to the fishery (Morin *et al.*, 2004), it may indicate that *S. fasciatus* could become a significant species in the future of the fishery.

4.5.7 Perspectives

Recent technical progresses in the analysis of archived otoliths (e.g. Hutchinson *et al.*, 1999) give access to an outstanding wealth of information for studying many unresolved issues regarding the genetic structure of past populations. Studying the genetic composition of the specimens that have contributed to the historical pulses of recruitment in the Northwest Atlantic would be an interesting step for inferring the status and the connectivity of redfish fishery stocks. This method would also provide valuable information about a possible loss of genetic diversity in the depleted redfish populations. Maintaining genetic diversity in a population is of prime importance for long-term adaptation and evolution. Recent studies have suggested that a loss of genetic diversity may be more frequent than anticipated in depleted populations of marine fishes (e.g., Turner *et al.*, 2002; Hauser *et al.*, 2002; Hutchinson *et al.*, 2003).

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Table 4.1 Description of the samples: date, fishing zone, geographical location, depth (m), and mean length (cm) by sex. Each sample is monospecific based on microsatellites.

							Males			Females			TOT
Sample	**Date**	**NAFO**	**Longitude**	**Latitude**	**Depth**	**Mean**	**SD**	***N***	**Mean**	**SD**	***N***	***N***
Name		**Div.**	**(W)**	**(N)**	**(m)**	**Size (cm)**			**Size (cm)**			
S. fasciatus												
Northeast, outside GSL–LCH												
3L65	fall 2001	3L	47°16.6	46°51.8	404	20.9	1.2	17	22.9	3.7	7	**24**
3N23	fall 2001	3N	50°22.2	42°47.5	408	23.5	2.4	14	26.1	3.5	18	**32**
3O44	fall 2001	3O	52°56.0	44°11.6	408	20.4	0.9	18	20.8	1.2	14	**32**
3PS1	summer 2002	3PS	55°10.2	45°08.2	202	24.7	1.5	16	26.3	2.2	16	**32**
3PS138	fall 2002	3PS	56°01.8	44°51.9	448	24.4	2.8	8	27.3	2.3	24	**32**
3PS26	summer 2002	3PS	56°14.4	44°52.2	354	22.1	1.2	17	25.6	2.9	15	**32**
Inside GSL–LCH												
3PS114	summer 2002	3PS	56°50.2	47°19.4	241	26.0	5.1	9	29.1	6.7	23	**32**
3PS88b	fall 2002	3PS	57°27.3	47°07.6	234	27.5	2.2	15	30.1	4.0	14	**29**
4R107	summer 2001	4R	59°10.8	49°45.5	217	26.8	1.5	18	30.6	1.9	13	**31**
4VN67	summer 2002	4VN	58°19.7	45°55.0	219	26.1	2.8	14	31.6	3.2	18	**32**
4VS36	summer 2002	4VS	57°42.2	45°16.3	217	25.1	1.2	12	27.3	2.5	20	**32**
4R53	fall 2002	4R	59°44.3	48°17.8	248	23.7	1.6	12	26.6	2.4	20	**32**
4VN5	fall 2002	4VN	58°07.0	45°47.1	245	24.9	1.5	6	28.9	2.4	26	**32**
BonBay	spring 2002	4R	—	—	—	24.1	1.4	9	29.0	3.3	23	**32**
Southwest, outside GSL–LCH												
NS85	summer 2001	4X	65°19.5	43°00.0	153	22.7	2.0	10	25.3	3.7	22	**32**
NS95	summer 2001	4W	60°03.2	43°27.4	504	27.5	1.3	7	34.1	2.9	25	**32**
s261	fall 2001	5Z	67°04.1	42°18.9	297	25.0	1.5	7	30.5	3.9	25	**32**
s266	fall 2002	5Z	69°54.3	42°18.7	203	22.3	1.1	20	26.3	1.6	12	**32**
s327	fall 2001	5Y	67°17.7	43°49.0	197	22.3	0.8	16	25.4	2.4	16	**32**
								245			**351**	**596**

Table 4.1 (continued)

Sample Name	Date		NAFO Div.	Longitude (W)	Latitude (N)	Depth (m)	Males Mean Size (cm)	Males SD	Males *N*	Females Mean Size (cm)	Females SD	Females *N*	TOT *N*
S. mentella													
Northeast, outside GSL–LCH													
2J42	fall	2001	2J	53°15.8	54°33.7	671	26.1	2.4	12	26.2	2.5	19	**31**
3L29	fall	2001	3L	47°25.4	48°03.1	506	24.4	2.1	10	24.9	1.7	18	**28**
s1050	fall	2001	1F	47°09.5	60°09.2	357	21.3	1.3	15	21.3	1.6	16	**31**
Inside GSL–LCH													
3PN1	summer	2002	3PN	58°50.0	46°53.3	434	29.1	1.5	21	30.2	2.1	11	**32**
3PN77	fall	2002	3PN	58°24.0	47°08.3	390	28.1	1.8	13	29.7	1.4	19	**32**
3PS133	fall	2002	3PS	56°49.2	45°32.0	394	29.3	2.8	13	30.8	1.8	18	**31**
4R48	summer	2002	4R	59°46.0	48°07.3	315	27.6	1.2	11	30.0	3.9	18	**29**
4R51	fall	2002	4R	59°58.9	48°19.1	392	30.3	1.4	18	32.7	1.0	14	**32**
4S35	fall	2002	4S	60°50.5	48°19.5	429	30.0	2.0	17	33.3	1.4	14	**31**
4S44	summer	2002	4S	60°34.2	48°03.6	447	29.7	2.2	15	31.4	2.0	16	**31**
4VN12	fall	2002	4VN	59°37.2	46°58.3	431	29.6	2.2	19	32.1	2.1	12	**31**
4VN2	fall	2002	4VN	57°53.0	45°42.8	425	29.3	1.9	15	31.7	1.5	17	**32**
4VN77	summer	2002	4VN	58°55.4	46°22.8	348	29.3	1.9	16	30.2	1.7	16	**32**
4VS13	summer	2002	4VS	58°02.8	44°13.8	515	30.0	1.6	15	31.7	1.6	15	**30**
4VS147	fall	2002	4VS	57°07.9	44°46.3	420	28.9	2.7	12	31.4	1.8	19	**31**
sag	winter	2003	SAG	—	—	—	25.5	0.8	19	26.0	1.1	12	**31**
									241			**254**	**495**
S. marinus													
3PN73	fall	2002	3PN	58°51.8	47°24.9	203	38.4	3.3	**19**	45.8	2.0	**11**	**30**
TOTAL									**505**			**616**	**1121**

Table 4.2 Descriptive statistics for each sample : sample size (N), number of alleles, number of private alleles, allelic richness, observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}), probability of heterozygote deficit (P). Global statistics at the end of the table are given as mean values within samples and as values over samples. The statistic F_{IT} is used to estimate the probability of heterozygote deficit over samples ($P_{(F_{IT})}$). Significant values after sequential Bonferroni correction are in bold characters.

	SEB25	**SEB31**	**SEB33**	**SEB9**	**SAL4**	**SEB30**	**SEB37**	**SEB46**	**SAL3**	**SEB45**	**SPI10II**	**SPI4II**	**SPI6**	**all loci**
S. fasciatus														
3L65														
N	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Nb. of alleles	14	2	16	6	6	14	11	9	4	9	18	14	7	10.0
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness (N = 24)	14.0	2.0	16.0	6.0	6.0	14.0	11.0	9.0	4.0	9.0	18.0	14.0	7.0	10.0
H_o	0.833	0.458	0.667	0.667	0.750	0.792	0.958	0.708	0.500	0.667	0.833	0.917	0.708	0.728
H_e	0.895	0.488	0.926	0.726	0.729	0.919	0.890	0.784	0.490	0.677	0.906	0.913	0.676	0.767
F_{IS}	0.058	0.063	**0.283**	0.083	-0.030	0.137	-0.078	0.091	-0.020	0.016	0.080	-0.009	-0.085	0.052
P	0.286	0.548	<0.001	0.307	0.693	0.042	0.949	0.230	0.639	0.550	0.172	0.672	0.841	0.027
3N23														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	13	2	15	6	8	19	12	11	3	7	18	17	8	10.7
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness (N = 24)	11.8	2.0	14.3	5.7	7.2	16.2	11.9	10.1	2.9	6.7	16.2	15.5	7.4	9.8
H_o	0.844	0.281	0.844	0.688	0.719	0.875	0.875	0.750	0.438	0.688	0.813	0.906	0.813	0.733
H_e	0.874	0.424	0.916	0.669	0.659	0.902	0.900	0.815	0.449	0.663	0.896	0.898	0.743	0.749
F_{IS}	0.024	0.340	0.080	-0.029	-0.122	0.030	0.026	0.081	-0.026	-0.066	0.094	-0.009	-0.096	0.022
P	0.440	0.068	0.117	0.669	0.938	0.390	0.418	0.200	0.662	0.827	0.092	0.662	0.894	0.189
3O44														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	14	2	16	10	7	23	12	10	3	9	18	16	10	11.5
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness (N = 24)	12.4	2.0	14.3	8.6	6.7	20.4	11.4	9.5	2.8	7.7	16.6	14.7	9.2	10.5
H_o	0.844	0.219	0.844	0.656	0.813	0.813	0.938	0.719	0.375	0.406	0.938	0.781	0.719	0.697
H_e	0.837	0.479	0.826	0.687	0.725	0.941	0.900	0.807	0.378	0.630	0.928	0.899	0.756	0.745
F_{IS}	-0.008	0.516	-0.021	0.025	-0.123	0.136	-0.043	0.097	-0.061	0.340	-0.010	0.126	0.050	0.065
P	0.640	0.007	0.725	0.488	0.927	0.010	0.857	0.155	0.780	0.003	0.690	0.039	0.348	0.004

Table 4.2 (continued)

	SEB25	SEB31	SEB33	SEB9	SAL4	SEB30	SEB37	SEB46	SAL3	SEB45	SPI10II	SPI4II	SPI6	all loci
3PS1														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	15	4	14	8	7	15	14	10	3	9	20	17	8	11.1
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness ($N = 24$)	14.0	3.7	13.5	7.4	6.4	14.1	13.4	9.4	3.0	8.4	17.8	14.9	7.7	10.3
H_o	0.781	0.438	0.781	0.656	0.781	0.813	0.844	0.781	0.594	0.781	0.938	0.906	0.750	0.757
H_e	0.867	0.560	0.914	0.752	0.738	0.916	0.907	0.783	0.550	0.686	0.936	0.876	0.800	0.787
F_{IS}	0.099	0.197	0.137	0.129	-0.060	0.115	0.071	0.002	-0.082	-0.141	-0.003	-0.036	0.041	0.039
P	0.096	0.145	0.025	0.125	0.802	0.043	0.162	0.581	0.780	0.977	0.627	0.809	0.397	0.045
3PS138														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	14	3	19	7	8	19	10	10	3	7	21	20	8	11.5
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness ($N = 24$)	13.3	2.9	16.5	6.4	7.4	17.6	9.7	9.4	3.0	6.4	18.4	17.8	7.4	10.5
H_o	0.844	0.406	0.875	0.781	0.844	0.813	0.781	0.813	0.406	0.656	0.875	0.969	0.781	0.757
H_e	0.878	0.520	0.890	0.688	0.740	0.927	0.883	0.785	0.461	0.626	0.931	0.929	0.720	0.764
F_{IS}	0.040	0.222	0.009	-0.138	-0.155	0.125	0.110	-0.035	0.075	-0.049	0.055	-0.044	-0.086	0.009
P	0.353	0.119	0.528	0.930	0.977	0.022	0.084	0.769	0.377	0.750	0.213	0.915	0.879	0.374
3PS26														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	15	3	12	7	8	19	11	9	3	8	22	14	8	10.7
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness ($N = 24$)	13.9	2.8	11.6	6.4	7.0	16.7	10.9	8.4	3.0	7.7	19.6	12.9	7.6	9.9
H_o	0.844	0.344	0.781	0.594	0.625	0.719	0.906	0.875	0.438	0.781	0.938	0.813	0.656	0.716
H_e	0.882	0.488	0.893	0.702	0.689	0.915	0.884	0.803	0.493	0.712	0.938	0.896	0.749	0.769
F_{IS}	0.042	0.298	0.127	0.141	0.065	0.216	-0.025	-0.094	0.113	-0.099	0.001	0.095	0.108	0.069
P	0.332	0.054	0.039	0.144	0.328	0.001	0.746	0.937	0.270	0.924	0.608	0.105	0.164	0.002

Table 4.2 (continued)

	SEB25	SEB31	SEB33	SEB9	SAL4	SEB30	SEB37	SEB46	SAL3	SEB45	SPI10II	SPI4II	SPI6	all loci
3PS114														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	16	2	20	11	6	21	15	10	4	9	20	15	10	12.2
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness ($N = 24$)	14.7	2.0	17.5	9.9	5.9	18.1	13.9	9.4	3.7	7.7	18.5	14.3	9.3	11.1
H_o	0.844	0.344	0.813	0.656	0.688	0.844	0.875	0.750	0.563	0.688	0.875	0.875	0.719	0.733
H_e	0.910	0.513	0.899	0.769	0.808	0.933	0.918	0.790	0.491	0.627	0.928	0.925	0.771	0.783
F_{IS}	0.074	0.310	0.088	0.134	0.140	0.095	0.047	0.035	-0.201	-0.098	0.051	0.052	0.069	0.065
P	0.150	0.076	0.107	0.098	0.089	0.061	0.273	0.416	0.958	0.893	0.234	0.239	0.266	0.002
3PS88b														
N	29	29	29	29	29	29	29	29	29	29	29	29	29	29
Nb. of alleles	18	6	21	9	6	19	13	11	3	11	23	16	9	12.7
Nb. of private alleles	-	-	2	-	-	1	-	-	-	-	-	1	-	4
Allelic richness ($N = 24$)	16.8	5.5	19.2	8.5	5.7	17.8	12.5	10.3	3.0	10.1	20.9	14.7	8.6	11.8
H_o	0.862	0.517	0.931	0.690	0.655	0.862	0.966	0.793	0.414	0.690	0.897	0.828	0.724	0.756
H_e	0.873	0.596	0.930	0.722	0.744	0.921	0.901	0.801	0.492	0.732	0.939	0.884	0.776	0.786
F_{IS}	-0.001	0.086	-0.005	0.045	0.110	0.064	-0.073	0.010	0.119	0.036	0.043	0.065	0.067	0.038
P	0.623	0.354	0.652	0.411	0.201	0.193	0.957	0.555	0.305	0.436	0.278	0.230	0.303	0.060
4R107														
N	31	31	31	31	31	31	31	31	31	31	31	31	31	31
Nb. of alleles	13	8	18	6	6	19	13	12	4	6	19	16	10	11.5
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness ($N = 24$)	12.5	7.4	16.5	6.0	5.8	17.0	11.6	10.8	4.0	5.3	17.0	14.7	9.7	10.6
H_o	0.871	0.484	0.806	0.774	0.645	0.871	0.839	0.839	0.516	0.581	0.839	0.903	0.710	0.744
H_e	0.912	0.536	0.918	0.717	0.742	0.919	0.848	0.804	0.467	0.564	0.925	0.866	0.829	0.770
F_{IS}	0.041	0.097	0.118	-0.082	0.133	0.051	0.011	-0.043	-0.121	-0.069	0.092	-0.044	0.146	0.033
P	0.316	0.264	0.040	0.860	0.134	0.246	0.529	0.795	0.887	0.796	0.081	0.856	0.055	0.074

Table 4.2 (continued)

	SEB25	SEB31	SEB33	SEB9	SAL4	SEB30	SEB37	SEB46	SAL3	SEB45	SPI10II	SPI4II	SPI6	all loci
4VN67														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	18	3	19	9	7	20	16	13	4	9	21	16	8	12.5
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness (N = 24)	16.3	2.8	16.8	8.4	6.2	17.8	14.3	11.4	3.7	7.9	18.9	15.0	7.7	11.3
H_o	0.813	0.375	0.875	0.781	0.688	0.875	0.938	0.719	0.469	0.688	0.906	0.844	0.750	0.748
H_e	0.920	0.507	0.913	0.765	0.701	0.927	0.884	0.682	0.516	0.597	0.921	0.916	0.783	0.768
F_{IS}	0.119	0.246	0.032	-0.021	0.019	0.052	-0.061	-0.055	0.054	-0.155	0.015	0.074	0.043	0.027
P	0.036	0.091	0.374	0.676	0.496	0.225	0.910	0.836	0.422	0.959	0.484	0.144	0.380	0.126
4VS36														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	14	6	19	6	6	18	11	11	4	13	20	17	7	11.7
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness (N = 24)	13.3	5.2	16.6	5.9	5.7	15.3	10.0	10.2	3.8	11.4	17.1	15.5	6.7	10.5
H_o	0.875	0.500	0.750	0.750	0.750	0.719	0.875	0.875	0.594	0.688	0.813	0.844	0.719	0.750
H_e	0.880	0.537	0.921	0.712	0.702	0.889	0.860	0.776	0.534	0.704	0.898	0.906	0.706	0.767
F_{IS}	0.005	0.033	0.185	-0.054	-0.074	0.191	-0.018	-0.130	-0.159	0.024	0.096	0.067	-0.018	0.023
P	0.561	0.482	0.003	0.775	0.832	0.004	0.687	0.985	0.928	0.471	0.080	0.176	0.650	0.170
4R53														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	13	3	18	7	5	17	10	10	4	9	16	14	9	10.4
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness (N = 24)	12.1	3.0	15.9	6.7	4.7	14.5	9.7	9.4	3.7	8.4	14.4	13.1	8.7	9.6
H_o	0.906	0.500	0.906	0.531	0.594	0.750	0.906	0.750	0.469	0.688	0.875	0.844	0.781	0.731
H_e	0.858	0.554	0.881	0.750	0.636	0.896	0.888	0.789	0.505	0.733	0.890	0.889	0.757	0.764
F_{IS}	-0.057	0.047	-0.035	0.283	0.068	0.161	-0.021	0.047	0.015	0.063	0.017	0.043	-0.033	0.045
P	0.868	0.462	0.806	0.005	0.367	0.016	0.715	0.357	0.540	0.294	0.484	0.314	0.745	0.035

Table 4.2 (continued)

	SEB25	SEB31	SEB33	SEB9	SAL4	SEB30	SEB37	SEB46	SAL3	SEB45	SPI10II	SPI4II	SPI6	all loci
4VN5														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	17	5	15	7	5	18	13	10	3	6	15	16	5	10.4
Nb. of private alleles	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Allelic richness ($N = 24$)	14.8	4.3	13.7	6.6	4.7	15.8	12.1	9.7	2.9	5.2	13.9	14.7	4.8	9.5
H_o	0.813	0.188	0.906	0.844	0.625	0.688	0.906	0.875	0.406	0.313	0.844	0.969	0.563	0.688
H_e	0.836	0.350	0.888	0.747	0.667	0.896	0.866	0.816	0.517	0.440	0.909	0.906	0.645	0.723
F_{IS}	0.028	0.461	-0.021	-0.147	0.064	0.233	-0.050	-0.073	0.187	0.253	0.072	-0.070	0.106	0.050
P	0.428	0.005	0.728	0.962	0.354	0.001	0.859	0.908	0.160	0.051	0.159	0.964	0.225	0.018
BonBay														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	13	3	12	8	6	9	9	11	4	7	11	15	6	8.8
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness ($N = 24$)	11.6	2.8	10.4	7.4	5.7	8.4	8.4	9.5	3.9	6.0	10.1	12.7	5.9	7.9
H_o	0.906	0.125	0.750	0.625	0.750	0.750	0.656	0.781	0.656	0.344	0.719	0.844	0.844	0.673
H_e	0.810	0.276	0.788	0.697	0.794	0.773	0.758	0.785	0.515	0.448	0.756	0.850	0.807	0.689
F_{IS}	-0.121	0.503	0.049	0.092	0.041	0.031	0.134	-0.003	-0.279	0.194	0.051	-0.003	-0.057	0.024
P	0.979	0.005	0.358	0.252	0.402	0.445	0.085	0.589	0.990	0.085	0.345	0.595	0.801	0.194
NS85														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	14	3	21	7	7	13	15	10	3	10	21	14	8	11.2
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness ($N = 24$)	13.2	2.8	18.5	6.4	6.4	11.4	13.8	9.1	2.9	8.9	18.3	12.6	7.7	10.2
H_o	0.781	0.438	0.781	0.625	0.781	0.750	0.844	0.781	0.594	0.594	0.875	0.781	0.844	0.728
H_e	0.876	0.479	0.934	0.771	0.671	0.862	0.906	0.785	0.543	0.540	0.927	0.875	0.765	0.755
F_{IS}	0.102	0.045	0.158	0.168	-0.168	0.125	0.064	-0.020	-0.132	-0.102	0.057	0.102	-0.105	0.036
P	0.092	0.443	0.004	0.067	0.955	0.074	0.203	0.674	0.839	0.951	0.205	0.111	0.933	0.068

Table 4.2 (continued)

	SEB25	SEB31	SEB33	SEB9	SAL4	SEB30	SEB37	SEB46	SAL3	SEB45	SPI10II	SPI4II	SPI6	all loci
NS95														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	15	2	15	5	7	19	12	12	3	9	24	19	9	11.6
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness ($N = 24$)	13.8	2.0	13.2	5.0	6.4	16.5	11.6	10.8	3.0	8.1	20.7	16.7	8.2	10.5
H_o	0.875	0.344	0.750	0.625	0.750	0.813	0.906	0.719	0.375	0.781	0.938	0.875	0.719	0.728
H_e	0.855	0.396	0.873	0.657	0.692	0.906	0.892	0.774	0.462	0.745	0.938	0.869	0.765	0.754
F_{IS}	-0.024	0.135	0.143	0.050	-0.085	0.104	-0.021	0.056	0.190	-0.055	-0.005	-0.007	0.061	0.034
P	0.731	0.371	0.034	0.408	0.871	0.064	0.728	0.326	0.149	0.777	0.656	0.652	0.316	0.078
s261														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	13	4	18	10	6	18	13	12	3	7	22	18	8	11.7
Nb. of private alleles	-	1	-	-	-	1	-	1	-	-	-	-	-	3
Allelic richness ($N = 24$)	12.1	3.5	17.1	9.1	5.6	15.6	11.6	10.7	3.0	6.2	19.3	16.5	7.7	10.6
H_o	0.875	0.375	0.875	0.531	0.656	0.906	0.844	0.844	0.344	0.531	0.781	0.906	0.844	0.716
H_e	0.872	0.537	0.941	0.762	0.675	0.875	0.846	0.794	0.448	0.571	0.922	0.927	0.808	0.763
F_{IS}	-0.014	0.263	0.069	0.303	0.014	-0.037	0.002	-0.064	0.235	0.071	0.153	0.022	-0.046	0.062
P	0.683	0.075	0.127	0.001	0.529	0.803	0.571	0.852	0.093	0.325	0.009	0.430	0.793	0.002
s266														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	14	2	17	7	7	14	16	10	4	6	15	18	8	10.6
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness ($N = 24$)	13.3	2.0	15.2	6.4	6.7	12.6	14.8	8.9	3.7	5.5	13.8	15.9	7.5	9.7
H_o	0.875	0.438	0.875	0.656	0.531	0.781	0.938	0.781	0.500	0.563	0.719	0.875	0.656	0.707
H_e	0.903	0.537	0.906	0.738	0.665	0.873	0.927	0.744	0.554	0.524	0.895	0.918	0.770	0.759
F_{IS}	0.031	0.137	0.035	0.112	0.175	0.107	-0.016	-0.051	0.067	-0.074	0.198	0.048	0.145	0.070
P	0.368	0.330	0.350	0.179	0.073	0.093	0.723	0.793	0.398	0.835	0.003	0.256	0.082	0.002

Table 4.2 (continued)

	SEB25	SEB31	SEB33	SEB9	SAL4	SEB30	SEB37	SEB46	SAL3	SEB45	SPI10II	SPI4II	SPI6	all loci
s327														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	16	3	17	5	6	16	13	11	5	8	24	16	11	11.6
Nb. of private alleles	-	-	-	-	-	-	-	-	1	-	1	-	-	2
Allelic richness ($N = 24$)	14.7	2.8	15.7	4.8	5.5	13.4	12.6	10.1	4.5	6.9	20.3	14.6	9.7	10.4
H_o	0.813	0.438	0.750	0.688	0.813	0.844	0.813	0.688	0.594	0.594	0.750	0.844	0.719	0.719
H_e	0.869	0.521	0.908	0.745	0.660	0.846	0.912	0.806	0.566	0.527	0.938	0.920	0.806	0.766
F_{IS}	0.066	0.131	0.171	0.078	-0.236	0.002	0.097	0.136	-0.049	-0.128	0.197	0.081	0.109	0.063
P	0.214	0.279	0.007	0.276	0.997	0.572	0.091	0.069	0.702	0.947	0.001	0.115	0.124	0.005
S. mentella														
2J42														
N	31	31	31	31	31	31	31	31	31	31	31	31	31	31
Nb. of alleles	14	14	24	8	3	24	18	12	4	16	23	9	10	13.8
Nb. of private alleles	-	1	-	-	-	-	2	-	-	-	-	-	1	4
Allelic richness ($N = 24$)	13.0	12.6	21.6	7.3	3.0	21.4	16.7	11.0	4.0	14.7	20.8	8.9	9.7	12.7
H_o	0.903	0.774	0.871	0.613	0.194	0.839	0.484	0.548	0.581	0.935	0.742	0.774	0.774	0.695
H_e	0.843	0.847	0.950	0.735	0.212	0.942	0.930	0.760	0.574	0.906	0.957	0.837	0.845	0.788
F_{IS}	-0.073	0.087	0.084	0.163	-0.062	0.111	**0.479**	0.280	-0.050	-0.041	**0.218**	0.063	0.083	**0.120**
P	0.937	0.174	0.063	0.070	1.000	0.025	<0.001	0.001	0.729	0.842	<0.001	0.267	0.186	<0.001
3L29														
N	28	28	28	28	28	28	28	28	28	28	28	28	28	28
Nb. of alleles	15	12	28	8	3	18	18	10	6	18	18	10	8	13.2
Nb. of private alleles	-	-	-	-	-	1	2	-	-	2	-	-	-	5
Allelic richness ($N = 24$)	14.2	11.8	26.1	7.7	3.0	16.5	17.1	9.1	5.8	16.9	17.3	9.6	8.0	12.6
H_o	0.893	0.786	0.964	0.714	0.179	0.786	0.679	0.357	0.393	0.893	0.893	0.821	0.714	0.698
H_e	0.888	0.845	0.974	0.723	0.262	0.904	0.934	0.594	0.456	0.924	0.914	0.871	0.855	0.771
F_{IS}	-0.017	0.070	0.007	0.013	0.229	0.126	**0.273**	0.376	0.088	0.022	0.023	0.058	0.163	**0.096**
P	0.719	0.230	0.559	0.540	0.171	0.048	<0.001	0.001	0.317	0.459	0.434	0.280	0.041	<0.001

Table 4.2 (continued)

	SEB25	SEB31	SEB33	SEB9	SAL4	SEB30	SEB37	SEB46	SAL3	SEB45	SPI10II	SPI4II	SPI6	all loci
s1050														
N	31	31	31	31	31	31	31	31	31	31	31	31	31	31
Nb. of alleles	10	12	24	9	4	22	22	8	5	14	10	10	11	12.4
Nb. of private alleles	-	-	-	-	-	1	-	-	-	-	1	-	-	2
Allelic richness ($N = 24$)	9.3	11.4	21.5	8.2	3.7	19.0	19.2	7.5	4.8	12.5	9.0	9.5	10.5	11.2
H_o	0.839	0.839	0.871	0.548	0.226	0.968	0.581	0.452	0.677	0.871	0.548	0.968	0.903	0.715
H_e	0.797	0.818	0.956	0.657	0.240	0.880	0.928	0.706	0.681	0.855	0.755	0.876	0.869	0.765
F_{IS}	-0.053	-0.026	0.090	0.163	-0.071	-0.102	**0.375**	0.348	0.006	-0.029	0.265	-0.106	-0.040	0.067
P	0.805	0.738	0.046	0.072	1.000	0.988	<0.001	0.001	0.556	0.739	0.004	0.986	0.804	0.002
3PN1														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	15	13	23	10	4	20	15	13	5	16	12	13	9	12.9
Nb. of private alleles	-	-	-	-	-	-	-	1	-	-	-	-	-	1
Allelic richness ($N = 24$)	13.9	11.8	20.5	9.7	3.8	17.2	14.1	11.9	4.9	14.7	10.6	12.1	8.2	11.8
H_o	0.906	0.813	0.844	0.906	0.406	0.688	0.688	0.719	0.750	0.938	0.781	0.875	0.813	0.779
H_e	0.890	0.758	0.954	0.800	0.511	0.890	0.925	0.860	0.663	0.903	0.787	0.884	0.850	0.818
F_{IS}	-0.026	-0.073	0.113	-0.136	0.172	0.229	0.253	0.165	-0.134	-0.039	0.007	0.010	0.044	0.048
P	0.757	0.904	0.017	0.989	0.138	0.001	0.001	0.020	0.914	0.850	0.546	0.525	0.350	0.010
3PN77														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	15	13	27	9	4	21	20	13	5	12	15	13	11	13.7
Nb. of private alleles	-	-	-	-	-	-	-	2	-	-	-	-	-	2
Allelic richness ($N = 24$)	13.8	11.9	23.8	8.4	3.9	18.5	17.6	11.7	5.0	10.4	13.1	12.1	10.2	12.3
H_o	0.938	0.750	0.938	0.656	0.594	0.938	0.844	0.813	0.719	0.906	0.781	0.875	0.875	0.817
H_e	0.885	0.789	0.961	0.705	0.494	0.930	0.903	0.832	0.680	0.836	0.807	0.888	0.886	0.811
F_{IS}	-0.060	0.047	0.025	0.067	-0.206	-0.010	0.066	0.019	-0.084	-0.100	0.018	0.013	0.009	-0.008
P	0.917	0.339	0.361	0.285	0.987	0.695	0.174	0.482	0.834	0.945	0.505	0.513	0.531	0.685

Table 4.2 (continued)

	SEB25	SEB31	SEB33	SEB9	SAL4	SEB30	SEB37	SEB46	SAL3	SEB45	SPI10II	SPI4II	SPI6	all loci
3PS133														
N	31	31	31	31	31	31	31	31	31	31	31	31	31	31
Nb. of alleles	15	12	25	9	3	22	17	13	6	16	12	15	8	13.3
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allelic richness ($N = 24$)	13.9	11.0	22.6	8.5	3.0	19.1	15.8	11.6	5.5	14.3	11.1	14.1	7.8	12.2
H_o	0.903	0.774	0.968	0.774	0.452	0.871	0.710	0.839	0.645	0.968	0.645	0.968	0.677	0.784
H_e	0.868	0.736	0.958	0.701	0.418	0.905	0.910	0.850	0.667	0.897	0.684	0.903	0.833	0.792
F_{IS}	-0.041	-0.053	-0.011	-0.107	-0.081	0.038	0.220	0.014	0.013	-0.085	0.057	-0.073	0.176	0.010
P	0.815	0.821	0.748	0.937	0.808	0.320	0.001	0.512	0.536	0.976	0.330	0.959	0.035	0.330
4R48														
N	29	29	29	29	29	29	29	29	29	29	29	29	29	29
Nb. of alleles	15	12	26	9	4	23	14	12	5	16	11	14	10	13.2
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Allelic richness ($N = 24$)	13.6	11.6	23.5	8.8	3.8	20.7	13.3	11.1	5.0	14.7	10.3	13.4	9.5	12.3
H_o	0.931	0.759	0.897	0.759	0.448	0.931	0.828	0.690	0.690	0.828	0.655	0.931	0.897	0.788
H_e	0.854	0.815	0.961	0.806	0.473	0.928	0.867	0.825	0.740	0.883	0.731	0.901	0.831	0.811
F_{IS}	-0.092	0.070	0.067	0.060	0.000	-0.005	0.041	0.148	0.070	0.063	0.080	-0.034	-0.080	0.029
P	0.964	0.237	0.106	0.301	0.589	0.664	0.366	0.056	0.320	0.233	0.243	0.805	0.909	0.085
4R51														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	13	11	24	9	3	20	21	12	7	12	10	15	10	12.8
Nb. of private alleles	-	-	-	-	-	-	2	1	-	-	-	-	-	3
Allelic richness ($N = 24$)	12.1	10.1	21.2	8.2	3.0	17.7	17.7	11.0	6.7	11.1	9.4	13.6	9.6	11.6
H_o	0.781	0.813	0.906	0.656	0.531	0.969	0.688	0.594	0.625	0.938	0.844	0.906	0.875	0.779
H_e	0.824	0.789	0.956	0.576	0.517	0.922	0.844	0.806	0.690	0.859	0.774	0.883	0.873	0.788
F_{IS}	0.050	-0.048	0.049	-0.142	-0.071	-0.051	0.187	0.253	0.096	-0.105	-0.093	-0.027	-0.003	0.012
P	0.318	0.811	0.189	0.980	0.764	0.938	0.006	0.002	0.236	0.974	0.929	0.758	0.603	0.298

Table 4.2 (continued)

	SEB25	SEB31	SEB33	SEB9	SAL4	SEB30	SEB37	SEB46	SAL3	SEB45	SPI10II	SPI4II	SPI6	all loci
4S35														
N	31	31	31	31	31	31	31	31	31	31	31	31	31	31
Nb. of alleles	13	13	25	9	4	16	18	11	5	15	11	12	8	12.3
Nb. of private alleles	-	-	-	-	-	-	2	-	-	-	-	-	-	2
Allelic richness (*N* = 24)	12.0	11.9	23.2	8.3	3.8	14.6	16.3	10.4	4.8	13.4	10.5	11.6	7.5	11.4
H_o	0.903	0.742	0.935	0.677	0.387	0.774	0.645	0.677	0.710	0.806	0.774	0.806	0.968	0.754
H_e	0.873	0.795	0.965	0.659	0.467	0.840	0.926	0.809	0.650	0.846	0.816	0.883	0.838	0.792
F_{IS}	-0.036	0.046	0.031	-0.033	0.119	0.079	**0.303**	0.157	-0.093	0.048	0.052	0.080	-0.158	0.048
P	0.784	0.365	0.306	0.739	0.262	0.177	<0.001	0.037	0.831	0.326	0.316	0.166	0.997	0.010
4S44														
N	31	31	31	31	31	31	31	31	31	31	31	31	31	31
Nb. of alleles	14	13	29	9	4	18	21	13	5	16	11	14	8	13.5
Nb. of private alleles	-	-	-	-	-	-	-	1	-	1	-	-	-	2
Allelic richness (*N* = 24)	12.4	11.7	25.5	8.7	3.8	15.9	18.9	12.0	5.0	14.5	10.2	13.2	7.5	12.2
H_o	0.806	0.742	0.968	0.645	0.516	0.774	0.839	0.613	0.710	0.839	0.806	0.935	0.839	0.772
H_e	0.823	0.778	0.965	0.680	0.602	0.884	0.925	0.833	0.678	0.852	0.774	0.889	0.834	0.804
F_{IS}	-0.003	0.030	-0.003	0.051	0.108	0.126	0.095	0.263	-0.047	0.016	-0.042	-0.055	-0.009	0.041
P	0.606	0.438	0.682	0.363	0.255	0.045	0.072	0.001	0.723	0.501	0.785	0.890	0.625	0.031
4VN12														
N	31	31	31	31	31	31	31	31	31	31	31	31	31	31
Nb. of alleles	16	13	23	10	5	20	18	12	6	11	10	13	9	12.8
Nb. of private alleles	1	-	-	-	-	-	-	-	-	-	-	-	-	1
Allelic richness (*N* = 24)	14.5	11.8	21.6	9.0	4.5	17.3	16.1	10.8	5.8	10.5	9.7	12.2	8.7	11.7
H_o	0.839	0.677	0.871	0.645	0.645	0.839	0.774	0.581	0.774	0.774	0.839	0.935	0.903	0.777
H_e	0.884	0.781	0.962	0.704	0.581	0.900	0.880	0.783	0.739	0.889	0.855	0.885	0.863	0.819
F_{IS}	0.048	0.118	0.095	0.085	-0.113	0.066	0.108	0.238	-0.048	0.125	0.019	-0.058	-0.048	0.052
P	0.303	0.103	0.028	0.243	0.874	0.205	0.078	0.007	0.747	0.062	0.476	0.898	0.825	0.008

Table 4.2 (continued)

	SEB25	SEB31	SEB33	SEB9	SAL4	SEB30	SEB37	SEB46	SAL3	SEB45	SPI10I1	SPI4I1	SPI6	all loci
4VN2														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	15	14	22	7	4	17	18	14	6	14	12	13	9	12.7
Nb. of private alleles	-	-	-	-	-	-	-	1	-	1	-	-	-	2
Allelic richness ($N = 24$)	13.6	12.6	20.3	6.9	3.9	15.1	16.1	12.7	5.7	12.1	10.9	12.1	8.7	11.6
H_o	0.969	0.813	0.938	0.719	0.563	0.781	0.719	0.750	0.750	0.781	0.750	0.875	0.969	0.798
H_e	0.876	0.857	0.954	0.738	0.571	0.886	0.918	0.810	0.707	0.831	0.839	0.878	0.860	0.822
F_{IS}	-0.108	0.052	0.017	0.027	0.015	0.118	0.219	0.075	-0.083	0.061	0.094	-0.003	-0.129	0.030
P	0.990	0.301	0.443	0.454	0.521	0.051	0.001	0.209	0.833	0.275	0.140	0.600	0.994	0.077
4VN77														
N	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Nb. of alleles	16	11	26	9	4	20	20	13	8	15	11	12	10	13.5
Nb. of private alleles	-	-	-	-	-	-	2	-	-	-	-	-	-	2
Allelic richness ($N = 24$)	13.9	9.9	23.4	8.8	3.8	17.3	17.7	11.9	7.4	13.3	10.2	11.4	9.6	12.2
H_o	0.906	0.719	0.969	0.813	0.469	0.750	0.875	0.688	0.688	0.875	0.719	0.969	0.906	0.796
H_e	0.884	0.773	0.962	0.721	0.583	0.898	0.929	0.818	0.774	0.862	0.768	0.890	0.846	0.818
F_{IS}	-0.033	0.069	-0.007	-0.129	0.155	0.160	0.059	0.158	0.113	-0.032	0.047	-0.090	-0.073	0.027
P	0.787	0.266	0.717	0.973	0.156	0.009	0.187	0.032	0.150	0.767	0.355	0.981	0.909	0.092
4VS13														
N	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Nb. of alleles	14	14	22	8	3	18	16	13	6	16	10	13	9	12.5
Nb. of private alleles	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Allelic richness ($N = 24$)	13.0	13.1	20.0	8.0	3.0	16.8	14.7	11.6	5.8	14.2	9.5	12.3	8.4	11.6
H_o	0.867	0.800	0.867	0.733	0.467	0.867	0.767	0.667	0.733	0.933	0.733	0.867	0.800	0.777
H_e	0.885	0.794	0.944	0.772	0.491	0.923	0.894	0.843	0.728	0.885	0.728	0.881	0.811	0.810
F_{IS}	0.008	-0.009	0.080	0.051	0.002	0.062	0.141	0.206	-0.007	-0.056	-0.009	0.017	0.014	0.042
P	0.543	0.660	0.094	0.344	0.572	0.186	0.034	0.009	0.608	0.884	0.644	0.485	0.508	0.026

Table 4.2 (continued)

	SEB25	SEB31	SEB33	SEB9	SAL4	SEB30	SEB37	SEB46	SAL3	SEB45	SPI10II	SPI4II	SPI6	all loci
4VS147														
N	31	31	31	31	31	31	31	31	31	31	31	31	31	31
Nb. of alleles	14	15	30	8	3	19	17	11	6	14	14	13	8	13.2
Nb. of private alleles	-	-	-	-	-	-	-	1	-	-	-	-	-	1
Allelic richness ($N = 24$)	12.7	13.4	26.3	7.5	3.0	16.9	15.7	10.3	5.8	12.5	12.4	12.2	7.8	12.0
H_o	1.000	0.742	0.968	0.516	0.452	0.774	0.710	0.742	0.645	0.839	0.871	0.935	0.839	0.772
H_e	0.876	0.809	0.969	0.717	0.513	0.888	0.907	0.855	0.682	0.844	0.865	0.892	0.851	0.815
F_{IS}	-0.145	0.065	0.002	0.262	0.122	0.121	0.209	0.133	0.028	0.006	-0.007	-0.049	0.015	0.054
P	1.000	0.269	0.625	0.009	0.252	0.051	0.002	0.056	0.475	0.549	0.625	0.870	0.491	0.005
sag														
N	31	31	31	31	31	31	31	31	31	31	31	31	31	31
Nb. of alleles	18	11	26	9	4	17	18	11	6	16	12	14	10	13.2
Nb. of private alleles	-	-	-	-	-	1	-	-	-	-	-	-	1	2
Allelic richness ($N = 24$)	16.0	10.2	23.4	8.2	3.8	15.0	16.4	10.4	5.8	14.3	10.9	12.8	9.1	12.0
H_o	0.903	0.742	1.000	0.645	0.419	0.839	0.516	0.774	0.548	0.903	0.710	0.871	0.710	0.737
H_e	0.892	0.695	0.953	0.644	0.470	0.865	0.922	0.792	0.735	0.827	0.809	0.899	0.854	0.792
F_{IS}	-0.013	-0.068	-0.050	-0.011	0.061	0.024	**0.437**	0.020	0.254	-0.094	0.115	0.032	0.166	0.070
P	0.682	0.872	1.000	0.636	0.390	0.448	<0.001	0.495	0.015	0.964	0.107	0.384	0.036	0.001
S. marinus														
3PN73														
N	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Nb. of alleles	12	11	18	12	4	18	16	16	5	12	12	17	9	12.5
Nb. of private alleles	-	1	2	-	-	3	-	1	-	2	-	-	-	9
Allelic richness ($N = 24$)	11.3	10.5	16.1	11.0	4.0	16.4	15.0	15.4	5.0	10.6	10.8	15.4	8.6	11.5
H_o	0.900	0.967	0.733	0.867	0.500	0.633	0.833	1.000	0.567	0.733	0.833	0.833	0.867	0.790
H_e	0.889	0.856	0.894	0.874	0.463	0.821	0.863	0.918	0.562	0.812	0.816	0.894	0.761	0.799
F_{IS}	-0.017	-0.140	0.175	0.001	-0.088	0.230	0.035	-0.092	-0.008	0.094	-0.031	0.069	-0.142	0.011
P	0.689	0.995	0.010	0.572	0.843	0.001	0.391	1.000	0.611	0.186	0.720	0.199	0.970	0.310

Table 4.2 (continued)

	SEB25	SEB31	SEB33	SEB9	SAL4	SEB30	SEB37	SEB46	SAL3	SEB45	SPI10II	SPI4II	SPI6	all loci
All 36 samples														
N_{TOT}	1121	1121	1121	1121	1121	1121	1121	1121	1121	1121	1121	1121	1121	1121
Tot. Nb. of alleles	23	21	44	17	8	56	43	30	9	33	40	32	19	28.8
Tot. Nb. of private alleles	1	3	4	-	-	9	10	9	1	6	2	1	4	50
Mean all. Rich. (N = 24)	15.3	10.4	21.5	8.8	5.4	21.8	16.8	12.0	5.2	12.3	16.2	15.5	9.2	13.1
F_{IS} (mean within samp.)	-0.002	**0.092**	0.064	0.045	-0.001	**0.089**	**0.102**	**0.073**	0.001	-0.009	**0.059**	0.011	0.011	**0.043**
P	0.575	<0.0001	<0.0001	0.003	0.516	<0.0001	<0.0001	<0.0001	0.469	0.749	<0.0001	0.128	0.216	<0.0001
H_o (over samples)	0.869	0.566	0.864	0.686	0.583	0.814	0.799	0.733	0.568	0.734	0.808	0.880	0.788	0.745
H_e (over samples)	0.908	0.796	0.945	0.816	0.693	0.948	0.924	0.859	0.673	0.784	0.896	0.912	0.838	0.846
F_{IT} (over samples)	**0.044**	**0.295**	**0.087**	**0.163**	**0.163**	**0.142**	**0.135**	**0.149**	**0.159**	**0.066**	**0.099**	**0.036**	**0.061**	**0.120**
$P_{(F_{IT})}$	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 4.3 Pairwise F_{ST} values (above diagonal) between *S. fasciatus* samples with significativity tests (below diagonal) :
(–) non significant, (+) significant before any correction, + significant after sequential Bonferroni correction

	3L65	3N23	3O44	3PS1	3PS138	3PS26	3PS114	3PS88b	4R107	4VN67	4VS36	4R53	4VN5	BonBay	NS85	NS95	s261	s266	s327
3L65		-0.002	0.006	0.001	-0.003	0.000	0.003	0.006	0.013	0.007	0.007	**0.016**	**0.019**	**0.052**	**0.016**	0.005	0.010	0.007	0.012
3N23	–		0.001	0.003	0.000	-0.003	0.004	0.004	0.008	0.006	0.004	**0.016**	**0.017**	**0.042**	**0.014**	0.002	0.005	0.005	0.011
3O44	–	–		0.001	-0.001	0.000	0.001	0.002	0.008	0.006	0.006	**0.014**	**0.016**	**0.044**	**0.017**	0.002	0.010	0.008	0.014
3PS1	–	–	–		0.000	-0.002	0.000	-0.003	0.006	0.004	-0.001	0.007	0.014	**0.039**	0.008	0.001	0.003	-0.001	0.001
3PS138	–	–	–	–		-0.003	-0.001	0.004	0.010	0.006	0.005	**0.017**	**0.017**	**0.047**	**0.019**	0.004	0.009	0.004	0.007
3PS26	–	–	–	–	–		0.002	0.004	0.010	0.008	0.002	0.012	**0.018**	**0.051**	**0.016**	0.004	0.003	0.001	0.006
3PS114	–	–	–	–	–	–		-0.004	0.002	-0.001	0.001	0.008	**0.017**	**0.038**	0.007	0.006	0.003	0.002	0.006
3PS88b	–	–	–	–	–	–	–		0.004	0.000	0.001	0.010	**0.023**	**0.041**	0.008	0.003	0.001	0.005	0.007
4R107	(+)	(+)	(+)	(+)	(+)	(+)	–	–		0.005	0.002	0.011	**0.014**	**0.035**	**0.013**	0.005	0.002	0.010	0.010
4VN67	–	(+)	–	–	(+)	(+)	–	–	–		0.001	**0.015**	**0.019**	**0.044**	0.010	0.008	0.005	0.003	0.009
4VS36	(+)	–	(+)	–	–	–	–	–	–	–		0.006	0.009	**0.038**	0.008	0.002	0.001	0.001	0.007
4R53	+	+	+	(+)	+	(+)	(+)	(+)	(+)	+	(+)		0.010	**0.038**	0.004	**0.015**	0.009	0.006	0.010
4VN5	+	+	+	(+)	+	+	+	+	+	+	(+)	(+)		**0.029**	0.007	**0.017**	**0.019**	0.010	0.012
BonBay	+	+	+	+	+	+	+	+	+	+	+	+	+		**0.032**	**0.042**	**0.046**	**0.041**	**0.038**
NS85	+	+	+	(+)	+	+	(+)	(+)	+	(+)	(+)	–	(+)	+		**0.019**	0.006	0.004	0.004
NS95	–	–	–	–	–	–	(+)	–	–	(+)	–	+	+	+	+		0.008	0.009	0.010
s261	(+)	–	(+)	–	(+)	–	–	–	–	–	–	(+)	+	+	–	(+)		0.000	0.001
s266	–	–	(+)	–	–	–	–	–	(+)	–	–	–	(+)	+	–	(+)	–		-0.005
s327	(+)	(+)	(+)	–	(+)	–	(+)	(+)	(+)	(+)	(+)	(+)	(+)	+	–	(+)	–	–	

Table 4.4 Pairwise F_{ST} values (above diagonal) between *S. mentella* samples with significativity tests (below diagonal) : (–) non significant, (+) significant before any correction, + significant after sequential Bonferroni correction

	2J42	3L29	s1050	3PN1	3PN77	3PS133	4R48	4R51	4S35	4S44	4VN12	4VN2	4VN77	4VS13	4VS147	sag
2J42		**0.012**	**0.027**	**0.039**	**0.033**	**0.043**	**0.041**	**0.041**	**0.040**	**0.037**	**0.039**	**0.034**	**0.043**	**0.040**	**0.033**	**0.042**
3L29	(+)		0.007	**0.025**	**0.018**	**0.027**	**0.027**	**0.025**	**0.022**	**0.020**	**0.025**	**0.021**	**0.024**	**0.022**	**0.019**	**0.025**
s1050	+	–		**0.016**	**0.012**	**0.014**	**0.016**	**0.018**	**0.012**	**0.012**	**0.016**	**0.015**	**0.014**	**0.013**	**0.013**	**0.015**
3PN1	+	+	+		-0.001	-0.003	0.001	0.007	-0.003	0.000	0.000	-0.002	-0.003	-0.001	-0.001	-0.001
3PN77	+	+	+	–		0.003	0.003	-0.001	0.001	0.000	0.001	-0.003	-0.001	0.001	-0.002	0.002
3PS133	+	+	+	–	–		0.004	0.007	0.002	0.003	0.004	0.003	0.002	0.000	0.005	-0.001
4R48	+	+	+	–	–	–		0.004	0.007	0.001	0.005	0.005	0.001	0.004	0.006	0.004
4R51	+	+	+	(+)	–	(+)	–		0.008	-0.001	0.003	0.004	0.005	0.006	0.006	0.004
4S35	+	+	+	–	–	–	(+)	(+)		-0.002	0.001	-0.002	-0.002	0.005	-0.001	-0.003
4S44	+	+	+	–	–	–	–	–	–		0.000	-0.002	-0.002	0.003	0.001	-0.001
4VN12	+	+	+	–	–	–	–	–	–	–		0.001	0.000	0.004	-0.001	0.001
4VN2	+	+	+	–	–	–	(+)	–	–	–	–		-0.001	0.003	0.001	0.003
4VN77	+	+	+	–	–	–	–	–	–	–	–	–		0.000	0.002	-0.002
4VS13	+	+	+	–	–	–	–	(+)	–	–	–	–	–		0.005	0.005
4VS147	+	+	+	–	–	–	(+)	(+)	–	–	–	–	–	–		0.002
sag	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	

Table 4.5 Differentiation among samples within species detailed by locus: mean within-sample heterozygosity (H_S), fixation index F_{ST} with the probability (P) of significance, and fixation index standardized for locus polymorphism (G'_{ST}; Hedrick, 2005). Significant values after sequential Bonferroni correction are in bold characters.

	S. mentella				*S. fasciatus*			
Locus	H_S	F_{ST}	P	G'_{ST}	H_S	F_{ST}	P	G'_{ST}
SEB25	0.861	**0.008**	<0.0001	0.061	0.872	**0.008**	<0.0001	0.057
SEB31	0.788	**0.010**	0.001	0.045	0.476	0.019	0.013	0.027
SEB33	0.958	0.001	0.290	0.000	0.896	**0.015**	<0.0001	0.131
SEB9	0.706	0.005	0.064	0.018	0.721	**0.012**	0.001	0.041
SAL4	0.445	**0.019**	<0.0001	0.033	0.701	0.009	0.005	0.028
SEB30	0.898	**0.012**	<0.0001	0.104	0.896	**0.014**	<0.0001	0.121
SEB37	0.907	0.007	0.057	0.034	0.881	**0.013**	<0.0001	0.106
SEB46	0.793	0.010	0.005	0.041	0.781	0.003	0.094	0.014
SAL3	0.671	**0.022**	<0.0001	0.067	0.483	-0.001	0.531	0.000
SEB45	0.864	**0.019**	<0.0001	0.140	0.612	**0.014**	<0.0001	0.035
SPI10II	0.799	**0.011**	0.001	0.047	0.911	**0.008**	0.001	0.083
SPI4II	0.882	-0.001	0.647	0.000	0.896	**0.011**	<0.0001	0.091
SPI6	0.848	0.007	0.005	0.049	0.755	0.006	0.021	0.026
over loci	0.801	0.009	<0.0001	0.042	0.760	**0.010**	<0.0001	0.039

CHAPITRE 5

CONCLUSION GÉNÉRALE

5.1 CONCLUSIONS

Chapitre 2 :

- La courbure de corps des poissons au moment de la capture des données morphométriques génère un artefact.
- L'artefact de courbure génère une variabilité plus importante que celle associée aux différences interspécifiques.
- L'artefact de courbure dépend uniquement de la façon dont le poisson est déposé sur son flanc au moment de la capture des points de repères. La courbure est indépendante de facteurs biologiques (taille, espèce, condition du poisson) et du mode de préservation des spécimens (congélation), mais pourrait être associée à la mort par suffocation.
- L'artefact de courbure peut être modélisé au moyen d'une analyse en composantes principales qui permet de définir un vecteur de déformation. La projection des données morphométriques perpendiculairement au vecteur de déformation, par la méthode de Burnaby, permet d'éliminer l'artefact du jeu de données.

Chapitre 3 :

- Les approches génétique et morphométrique permettent toutes deux de discriminer significativement les espèces.
- L'approche génétique permet de discriminer les espèces par méthode de groupement, sans information *a priori* (les critères usuels sont uniquement utilisés pour identifier les groupes générés par groupement). Par contraste, l'approche morphométrique nécessite une calibration du modèle discriminant à l'aide de l'information fournie par les microsatellites.
- Les données microsatellites et morphométriques individuelles montrent une corrélation significative, ce qui confirme l'hypothèse d'une forte détermination génétique de la forme des sébastes.
- Les résultats morphométriques confirment que *S. mentella* est davantage fusiforme que *S. fasciatus* pour la forme du corps.
- L'utilisation de 13 microsatellites, comparativement aux huit utilisés antérieurement, améliore la discrimination interspécifique.
- La corrélation négative observée, pour les espèces, entre le polymorphisme des loci et la valeur de l'indice de différenciation génétique F_{ST} indique que les valeurs de F_{ST} sont contraintes par le polymorphisme.
- Le recours à l'indice de différenciation G'_{ST} (standardisé pour l'effet du polymorphisme) permet de comparer les niveaux de différenciation exprimés par les différents loci.

- Les loci les plus discriminants entre les espèces (SEB30 et SEB31) sont composés d'un motif microsatellite interrompu. Il est proposé que le niveau d'homoplasie est moindre à ces loci.
- La méthode bayésienne de groupement appliquée aux données microsatellites confirme la présence d'hybridation introgressive asymétrique entre *S. mentella* et *S. fasciatus* et suggère la participation de *S. marinus* au processus d'hybridation introgressive.
- Les données morphométriques réfutent l'hypothèse que l'hybridation introgressive entraîne une convergence de la forme corporelle de *S. mentella* et *S. fasciatus*, ce qui contredit des observations antérieures. Il ressort que la convergence observée antérieurement reflétait un manque de puissance de la *MDH-A** utilisée comme critère d'assignation d'espèce.
- Les individus d'origine hybride, c'est-à-dire ceux combinant des allèles microsatellites typiques des deux espèces, ne présentent pas une plus grande variabilité de la forme corporelle que les individus génétiquement plus homogène pour des allèles spécifiques. Cette observation contredit l'hypothèse que l'hybridation introgressive augmente l'instabilité du développement des individus.
- Malheureusement, la tentative de documenter l'asymétrie fluctuante, qui est supposée refléter l'instabilité du développement, a été infructueuse. L'échec de la tentative découle en grande partie de la difficulté de trouver des variables qui respectent les conditions préalables d'une étude d'asymétrie fluctuante.

Chapitre 4 :

- Dans l'ensemble, les données microsatellites et morphométriques indiquent que la structure des populations est faible. Il existe toutefois une structure à grande échelle, à laquelle se superpose de l'hétérogénéité localisée.
- Les facteurs environnementaux à grande échelle contribuent à structurer génétiquement les populations à grande échelle (hypothèse du *member-vagrant*). Ils semblent promouvoir la plasticité phénotypique et participent éventuellement au processus de sélection morphologique adaptative.
- L'hybridation introgressive contribue à la structure des populations à grande échelle et à l'hétérogénéité locale.
- Pour les deux espèces, l'hybridation introgressive est restreinte au golfe du Saint-Laurent et au Chenal Laurentien.
- Pour *S. mentella*, les données génétiques et morphométriques convergent pour indiquer la présence d'une seule grande population dans le golfe du Saint-Laurent et le chenal Laurentien (GSL–CHL).
- La population de *S. mentella* du GSL–LCH est significativement différente des populations situées au nord du Grand Banc de Terre-Neuve (3L), dans la mer du Labrador (2J42) et à l'ouest du Groenland (1F).
- Pour *S. mentella*, les microsatellites indiquent une absence de différenciation entre le nord du Grand Banc (3L) et le Groenland (1F), mais un isolement de 2J (Labrador). La présence d'une population isolée dans le Labrador reste à confirmer, car cette observation repose sur un seul échantillon.

- Pour *S. mentella*, la morphométrie suggère une différenciation entre le Groenland (1F) et les eaux canadiennes (2J et 3L), mais ce résultat pourrait être influencé par de l'allométrie et reste à confirmer.
- L'apparente contradiction entre les résultats génétiques et morphométriques pour *S. mentella* des Grands Bancs (3L) et du Groenland (1F) pourrait être expliquée par un flux génique suffisant pour homogénéiser les fréquences génétiques entre les deux populations, associé à de la plasticité phénotypique induite par les différentes caractéristiques des eaux canadiennes et groenlandaises.
- La population du Saguenay est en majorité composée de l'espèce *S. mentella*. Cette population ne présente pas de perte de diversité génétique et n'est pas génétiquement différenciée de la population du GSL–LCH. La forme des spécimens est toutefois significativement différenciée dans le Saguenay.
- Les sébastes du Saguenay pourraient représenter une population puits (*sink*), dont les individus verraient leur forme modifiée par les conditions environnementales propres au Fjord. Cette hypothèse implique un certain degré de plasticité phénotypique.
- Dans l'ensemble, les microsatellites et la morphométrie suggèrent la présence de trois populations très faiblement différenciées de *S. fasciatus* à grande échelle : (i) autour des Grand Bancs de Terre-Neuve, jusqu'à l'extrémité sud du banc de Saint-Pierre, (ii) dans le Golf du Saint-Laurent et le Chenal Laurentien, et (iii) dans le golfe du Maine.
- Pour *S. fasciatus*, il existe de l'hétérogénéité génétique à l'intérieur du GSL–LCH. Cette hétérogénéité ne s'explique ni par un effet temporel, ni par différents niveaux d'hybridation introgressive, ni par de l'immigration. Elle suggère donc la présence de

populations locales, ce qui est difficilement explicable considérant le cycle de vie de l'espèce, la géographie et l'environnement.

- Les distances génétiques et la distribution des critères usuels (AFC, EGM, MDH) suggèrent que le golfe du Maine représente historiquement la population d'origine de *S. fasciatus* à partir de laquelle les autres populations ont colonisé les régions.
- La quasi absence d'individus génétiquement introgressés dans le golfe du Maine suggère l'absence de dérive larvaire à partir du golfe du Saint-Laurent, malgré des courants favorables, à moins que de la sélection ne soit impliquée.
- La faible différenciation du golfe du Maine pourrait s'expliquer par des épisodes d'immigration à partir des autres régions. Ces épisodes pourraient être influencés par les caractéristiques des masses d'eau.
- La population du fjord de Bonne Bay est composée de l'espèce *S. fasciatus*. Cette population présente une perte de diversité génétique et est génétiquement et morphologiquement significativement différente de la population du GSL–LCH.
- Les sébastes de Bonne Bay pourraient représenter une population isolée qui serait consécutive à un événement fondateur, suivi d'un flux génique limité. La forme particulière des sébastes de Bonne Bay serait le résultat de plasticité phénotypique ou d'adaptation sélective aux conditions environnementales locales.
- La structure des populations observée dans la zone de sympatrie n'est pas identique pour les deux espèces, ce qui complique les stratégies de gestion qui peuvent, dans la pratique, difficilement considérer les deux espèces séparément. L'établissement de

priorités de gestion tenant compte de la contribution relative des espèces à la pêche est problématique, car cette contribution relative pourrait varier.

5.2 PERSPECTIVES

Les techniques d'analyses moléculaires permettent désormais d'analyser l'ADN présent sur les otolithes archivés (e.g. Hutchinson *et al.*, 1999). Ce progrès donne accès à une impressionnante quantité d'information, jusqu'ici inexplorée. Dans le cas du sébaste, il serait possible de déterminer la composition génétique des individus ayant contribué aux pics historiques de recrutement, dans les différentes unités de gestion. Cette information permettrait de déterminer, pour les 50 dernières années, la contribution relative des espèces aux différents stocks de pêche et le degré de connectivité entre les stocks. De plus, cette information donnerait l'opportunité de vérifier si l'effondrement des stocks de pêche s'est accompagné d'une perte de diversité génétique. Le maintien de la diversité génétique est un facteur essentiel pour l'adaptation à long terme et l'évolution des populations. Des études récentes ont suggéré que la perte de diversité génétique pourrait être plus fréquente que prévu chez les populations réduites de poissons marins (ex., Turner *et al.*, 2002; Hauser *et al.*, 2002; Hutchinson *et al.*, 2003).

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