1   Anthropogenic boat noise reduces feeding success in winter flounder larvae

2   (*Pseudopleuronectes americanus*)

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Abstract

The aim of this study was to explore an emerging discipline addressing the impact of anthropogenic noise on larval stages of marine organisms. We assessed the influence of boat noise on the feeding behaviour of the pelagic larvae of winter flounder (*Pseudopleuronectes americanus*, Walbaum 1792). The hypothesis was that boat noise influences the feeding behaviour of *P. americanus* flounder larvae independently of prey density. Aquaria containing *P. americanus* larvae were placed in water baths in which boat noise was diffused for the "noise" treatment and compared to control aquaria with no sound emissions. Larvae were filmed using cameras placed above the aquaria and their behaviour was recorded. Larvae exposed to anthropogenic noise displayed significantly fewer hunting events than controls, and their stomach volumes were significantly smaller. This noise effect was the same at all prey densities used, suggesting that larval feeding behaviour is negatively impaired by anthropogenic noise.

Keywords: Feeding behaviour, winter flounder, larvae, anthropogenic noise, vessel noise

Introduction

Ambient underwater sound in the aquatic environment is used by marine organisms for orientation, communication among individuals, predator avoidance, and prey detection, and it can also be used as a larval settlement cue in various species, especially among those residing in coastal areas (Montgomery et al. 2006; Götz et al. 2009; Slabbekoorn et al. 2010; Stanley et al. 2010). However, anthropogenic underwater noise resulting from increasing maritime navigation, offshore wind and tidal turbines, mariculture facilities, piling installations, and seismic surveys for oil and gas exploration can have detrimental impacts on many marine organisms (Southall et al. 2007; Popper and Hastings 2009; Slabbekoorn et al., 2010; Popper et al. 2014; Kunc et al. 2016; Cox et al. 2018). These negative effects will likely increase since anthropogenic noise levels are rising with increasing human activities (Williams et al. 2015).

Studies on the effects of noise on foraging have been done with different life stages and different fish species, but studying larval stages is of special interest (high sensitivity to abiotic factors; the importance of larval survival in population recruitment) and poses specific challenges. Larval marine organisms have been shown to be sensitive to aquatic noise. Invertebrate larvae can detect and respond to aquatic noise (Simpson et al. 2011). Using the aquatic larval stage of damselfly, a freshwater invertebrate feeding on Daphnia during this aquatic phase, Villabolos-Jimenez et al. (2017) obtained results suggesting that underwater noise may decrease feeding. Anthropogenic noise may cause malformations or delays in development (seismic pulses, Aguilar de Soto et al. 2013; boat traffic noise,

59   Nedelec et al. 2014) as well as damage to sensory epithelia (sinusoidal wave sweeps, Solé

60   et al. 2016).

61   In larvae from four damselfish species (*Pomacentrus amboinensis*, *P. brachialis*, *P.*

62   *moluccensis*, *P. nagasakiensis*), Simpson et al. (2010) showed that orientation behaviour

63   is influenced by recent acoustic experience, i.e., reef noise. Positive orientation behaviour

64   in response to reef noise was also found in larvae of longspine cardinalfish (*Apogon*

65   *doryssa*) (Holles et al. 2013), but these authors showed that boat noise can disrupt this

66   behaviour. In Atlantic cod (*Gadus morhua*) larvae, the increased rate of ship noise

67   playbacks as well as "regular" ship noise caused a reduction in growth, while yolk sac

68   resorption was more rapid in the presence of "regular" noise (Nedelec et al. 2015). More

69   recently, Fakan and McCormick (2019) showed an increased heart rate during

70   embryogenesis in cinnamon clownfish (*Amphiprion melanopus*) and spiny chromis

71   (*Acanthochromis polyacanthus*) exposed to boat noise. This study also clearly

72   demonstrated that the effects of noise are species-specific.

73   In marine fishes, the larval stage is critical in terms of survival and is recognized as

74   being the main source of variability in the annual recruitment of feral populations (Houde

75   2016). Many factors determine larval survival rate including feeding success (Fortier and

76   Harris 1989; Robert et al. 2008), and aquaculture-related studies have shown that transition

77   to exogenous feeding once vitelline reserves are depleted seems to be particularly critical

78   (e.g., Øie et al. 2017).

79   Here, we used winter flounder, *Pseudopleuronectes americanus*, a species for which

80   reproduction and larval feeding are well understood (Audet and Tremblay 2011) to

81   determine how motorized boat noise combined with prey density would affect feeding

82   success during this critical stage. We hypothesized that anthropogenic vessel noise would

83   impair feeding success during feeding on live prey and that this effect would be more

84   pronounced with lower prey availability. Winter flounder is a dominant benthic species in

85   the St. Lawrence estuary (Bigelow and Schroeder 1953; Pereira et al. 1999) and is present

86   in areas where a local rise in ambient noise levels has been noted (Gervaise et al. 2012).

87   Winter flounder sport fishing is economically important in North America (Fairchild 2008;

88   Fishwatch 2019), including Québec (DFO, 2012). However, over the last few decades,

89   most populations including that of the St. Lawrence estuary have encountered marked

90   decreases for reasons that remain to be identified. Conservation issues thus require research

91   efforts on all factors that may affect recruitment. Like many marine vertebrates, *P.*

92   *americanus* has a complex life cycle. Upon hatching, larvae are pelagic until they undergo

93   metamorphosis to the juvenile stage (Pereira et al. 1999). During this pelagic larval phase,

94   feeding success is vital for growth and for accumulating enough energy for successful

95   metamorphosis. Larvae are visual feeders and spend most of the critical periods of their

96   development in shallow coastal areas, which are especially vulnerable to boat noise. Thus,

97   this species is likely a good model to assess the impact of anthropogenic noise on marine

98   species that use coastal areas during critical stages of their life history.

99

100   **Material and methods**

101

102   Larval production

103

104 Mature *P. americanus* females were captured in the Baie des Chaleurs offshore of

105 Bonaventure (QC, Canada) during the breeding season and brought to the Station aquicole

106 de l'ISMER (Rimouski, QC, Canada; 48° 31' N; 68° 28' W). Fertile males were available

107 at the wet lab facility. Egg fertilization and incubation were done according to the method

108 described by Vagner et al. (2013). Upon hatching, larvae were reared according to the

109 standard procedures used in our laboratory (Vagner et al. 2013; Martinez-Silva et al. 2018).

110 Briefly, larvae were transferred into 55 L cylindro-conical fiberglass tanks and reared in

111 green water at 10°C with a 12h:12h L:D photoperiod. Larval density was set to 1 larva

112 ml^{-1}, gentle aeration was used to create upwelling, and a constant flow of filtered (1 μm)

113 seawater was provided. In larval rearing tanks, the water supply was stopped each day for

114 12 h while a green water preparation (*Nannochloropsis oculata* at 1.6×10^6 cells L^{-1}) was

115 added to each tank. At the end of the day, water circulation was restored, allowing complete

116 renewal of the tank water overnight. Larvae were routinely co-fed with rotifers, *Brachionus*

117 *plicatilis*, and a commercial diet (GEMMA Wean 0.3, SKRETTING, France) was added

118 to their regime once they reached 5.5 mm (Ben Khemis et al. 2003; Vagner et al. 2013;

119 Martinez-Silva et al. 2018).

120

121 Underwater sound

122

123 As described in Jolivet et al. (2016), the vessel noise emitted during the experiments was

124 initially recorded at a mussel aquaculture site at St. Peter's Bay on Prince Edward Island

125 (Canada; 46° 25.963' N; 62° 39.914' W). The calibrated hydrophone (High Tech, Inc.,

126 Mississippi, USA, HTI-99-HF: sensitivity −169.7 dB re 1 V/µ Pa; frequency range 2 Hz to

127 125 kHz flat response) was placed 25 cm from the bottom, near the anchor of the mussel

128 line, and connected to an underwater acoustic recorder (RTSYS-Marine Technologies,

129 France, EA-SDA14, 156 kHz, 24-bit resolution). The farmer's boat (11 m in length; D &

130 H Boatbuilding hull with diesel motor, Cummins 300 hp C series) passed three times above

131 the recording hydrophone during calm natural conditions characterized by a wave height

132 of 0.2 m and wind speed of 3.8 m s^{-1} (http://climat.meteo.gc.ca/). From the recording, a

133 sequence lasting 30 s that corresponded to the maximum vessel noise sound intensity was

134 selected using customized codes written in MATLAB (The MathWorks, Inc.) software.

135

136 Experimental design

137

138 For the experiments, four 40 L aquaria were used, each containing 30 L of water. A

139 rectangular 2 L glass container was placed on a platform 19.5 cm from the aquarium's

140 bottom so that the upper rim of the aquarium was 1 cm above the water's surface (Fig. 1).

141 Water temperature in the aquaria was constant and monitored by probes (Onset Hobo

142 Water Temp Pro V2 Data logger U22-001); the average temperature was 12.1 ± 0.4°C with

143 a mean increase of 1.2 ± 0.2°C between the start and the end of experiments (2 h). Low-

144 intensity lights were installed above each aquarium to reproduce a light intensity present

145 in the production room (around 400 lux).

146 Twenty experimental larvae were randomly sampled in the water column of the rearing

147 tank prior to the morning feeding, which occurred when the lights were turned on in the

148 production room. Larvae were transferred into the 2 L containers (1.8 L of water) to

149 acclimate for 1 h prior to the start of the experiment and were not fed during the acclimation

150 to ensure feeding during the experiment. No boat noise was played during the acclimation

151 period. A digital camera (GoPro, Hero+ LCD CHDHB-401) fitted with a macro filter

152 (Polar Pro, macro filter 15-06746) was installed above each aquarium to record larval

153 behaviour for 2 h following the acclimation period (Fig. 1).

154 For each experimental trial, larval behaviour was monitored simultaneously in the

155 presence or absence of vessel noise; there were two aquaria per treatment and four

156 experimental trials were carried out. Individual larval behaviour was then monitored for 20

157 larvae × two aquaria × four trials, for a total of 160 larvae in the presence of boat noise and

158 160 larvae with no noise. Larvae could not see the sound source because the bottom of the

159 aquarium was opaque to facilitate their tracking. In the experimental tanks, larvae were

160 only offered rotifers, with no commercial diet. Prey density was low in the first trial ($5 \pm$

161 0.6 rotifers ml^{-1}), medium in the second and third trials (11 ± 2.0 rotifers ml^{-1}), and high in

162 the fourth trial (14 ± 0.8 rotifers ml^{-1}). The low and medium densities correspond to prey

163 densities previously used with success along with co-feeding with commercial diet (Vagner

164 et al. 2013; Martinez-Silva et al. 2018).

165

166

167 Sound emissions

168

169 Waterproof speakers (AQUA 30, DNH, 8 ohms, 80–20,000 Hz), plugged to an amplifier

170 (Plug & Play 12 W) and connected to a PC, were installed in two of the four aquaria to

171 continuously replay the vessel noise. The sound emission under experimental conditions

172 was calibrated using a calibrated hydrophone (HTI-96 MIN; High Tech, Inc.) associated

173 with a digital recorder (Song Meter SM2+; Wildlife Acoustics). The source was placed in

174 the center of the aquarium just below the glass container. The multiple reflections off the

175 sides of the aquaria produced homogeneous sound conditions (S.E.: ± 1.5 dB) in the glass

176 container, which was confirmed by sound measurements performed in each corner and the

177 center prior the experiment. A correction function was calculated from 30 s recordings of

178 calibrated sound done in each corner and the center, and this was applied to the vessel noise

179 to replicate the *in situ* spectrum of the vessel noise as nearly as possible. By varying the

180 gain of the amplifier, the intensity was adjusted to match natural conditions (sound level

181 [SL]: 130 dB re $1\mu Pa^2$ between 100 and 10,000 Hz). Two recordings were also made in

182 adjacent basins to check the "silent" conditions (located 1.5 m away).

183

184 Rotifer production and counts

185

186 Rotifers were reared in an 18 L tank and fed with Selco® S.parkle (INVE Aquaculture

187 Ltd., Thailand) four times a day. The culture concentration was estimated every morning,

188 and the volume of culture needed to reach a given prey concentration in each experimental

189 aquarium was calculated.

190   After the 1 h acclimation period, the video recording was started, rotifers (prey) were

191   added to each experimental aquarium, and seawater was added to top-up each aquarium to

192   2 L. At the end of the experiments, subsamples of rotifer cultures and seawater from each

193   aquarium were filtered through a 10 μm sieve and preserved in 95% ethanol for prey

194   concentration assessment. Rotifer concentration was estimated both before and after the

195   experiments by diluting the filtered rotifer samples in 80 ml of ethanol and counting rotifers

196   in 3 ml aliquots under a binocular microscope (three aliquots per sample).

197

198   Behaviour analysis

199

200   The videos were analyzed using Noldus Observer XT 9.0 software (Noldus Information

201   Technology Inc., Leesburg, VA, USA). A delimited field covering 95% of the aquarium's

202   bottom but avoiding sides and corners, where visibility was impaired, was used to analyze

203   the videos. Only larvae found within this delimited field were considered. A period of 12

204   minutes, always from minute 12 to 24 of the 2 h video for standardization purposes, was

205   analyzed for occurrence frequencies and duration of each behavioural trait (in seconds) for

206   each replicate and for each larva present in the observation field, giving a total of 132 larvae

207   in the absence and 117 in the presence of boat noise. The various observed and recorded

208   behaviours were set to be mutually exclusive state events except for hunting, which was

209   considered as a point event. The following behaviours were recorded: (1) Hunting: larva

210   moves rapidly with a wiggling movement; (2) Swimming: larva moves around actively in

211   the water column; (3) Resting: larva undertakes no active movement but rather stays

212 motionless on the bottom or floats passively; (4) Out: larva swims out of the observation

213 field.

214

215 Morphometric measurements

216

217 At the end of each experiment, all larvae were recovered and sacrificed by prolonged

218 anaesthesia in MS 222. A side-view photo was taken using a digital camera (Evolution VF,

219 Media Cybernetics) fixed on a binocular microscope at 20X magnification (Olympus SZ61

220 model SZ2-ST; Olympus Corporation, Tokyo, Japan) that was connected to a personal

221 computer (Fig. 2). A cold light source (NCL 150; Volpi, USA) was used to illuminate

222 specimens. For each individual, fork length (FL) and eye diameter (to check whether larvae

223 were of similar sizes) as well as stomach height, length, and surface area were measured

224 on each photo using image processing software (Image-Pro Express 5.1.0.12; Media

225 Cybernetics, Inc., USA). Stomach volume was calculated under the rough assumption of

226 cylindrical shape ($V = \pi\, h\, r^2$; h = stomach height; r = stomach radius).

227

228 Statistical analysis

229

230 To compare larvae at the same stage of development, it was necessary to do experiments

231 within a short period of time, which limited the number of trials that could be run. This

232 precluded the use of test aquarium as the statistical unit because there were relatively few

233 trials and thus low statistical power. However, the large volume of the experimental system

234   vs. the size of larvae was such that interactions between larvae were avoided. The video

235   analysis of 20 larvae per aquarium showed that larvae had no gregarious behaviour, and no

236   behavioural interactions were observed among individuals. For these reasons, larvae were

237   used as the statistical unit. Analyses were performed using STATISTICA software

238   (STATISTICA 6.1, StatSoft Inc., France). Normality and homoscedasticity were verified

239   using Kolmogorov-Smirnov and Levene tests, respectively (α =0.05). Two-way nested

240   ANOVAs (replicate aquaria nested in the "noise" factor) were used to determine significant

241   differences in larval feeding and swimming behaviour as a function of the presence or

242   absence of boat noise and prey concentration (low, medium, high). Because no significant

243   replicate effect was found for any of the variables tested ("aquarium" nested in "boat

244   noise"; $p > 0.05$ for all variables considered), two-way ANOVAs testing interactions

245   between factors (boat noise × prey density) were run. When significant factor effects were

246   found, *a posteriori* Tukey comparison of means tests ($\alpha = 0.05$) were used.

247

248   **Results**

249

250   Underwater sound recording

251

252   The replayed vessel noise in the two aquaria was homogenous, with 129 and 127

253   dB re 1µPa² between 100 and 1,000 Hz corresponding to the *in situ* recorded source signal

254   (Table 1, Fig. 3; aquaria 3 and 4). The sound levels measured in the two aquaria under

255   control conditions differed from the other two (Table 1, Fig. 3; aquaria 1 and 2). The sound

256 levels in the aquaria with no sound treatment were slightly higher than the conditions

257 before the experiments (Table 1) but remained consistent with natural conditions as defined

258 by Wenz's formula (Wenz 1962) (Fig. 3).

259

260 Predation analysis

261

262 Even though we used larvae of the same age, fork length and eye diameter differed slightly

263 among treatments (Fig. 4a, b). There was a significant boat noise × prey density effect

264 ($F_{2,312}$ = 3.65, p = 0.027) on larval fork length. However, the *a posteriori* test failed to find

265 a significant difference among treatments, and the overall mean larval fork length (± S.D.)

266 was 4.77 ± 0.515 mm. A significant boat noise × prey density effect ($F_{2,312}$ = 4.09,

267 p = 0.018) was also observed for eye diameter (Fig. 4b): larvae with the smallest eyes were

268 from the low prey density/absence of boat noise combination. As stated above, nested

269 ANOVA failed to indicate any differences between replicate aquaria for each experiment,

270 and this was also the case for fork length and eye diameter.

271 The variation in prey concentration had no effect on the hunting behaviour ($F_{2, 243}$ =

272 1.28, p = 0.28) while hunting attempts were 34% less frequent in the presence of sound

273 compared to the treatment when sound treatment was absent ($F_{1, 243}$ = 6.75, p = 0.010; Fig.

274 5a). There was no interaction between prey concentration and sound treatment ($F_{2, 243}$ =

275 1.71, p = 0.18) on the number of hunts. Stomach volume measurements (Fig. 5b) showed

276 that stomachs were 13% smaller in the presence of boat noise ($F_{1, 312}$ = 4.29, p = 0.039),

277 and this effect was independent of prey density (boat noise × prey density, $F_{2, 312} = 0.488$,

278 $p = 0.614$).

279   Prey concentration significantly influenced some of the measured variables, but without

280 interaction effects with the presence or absence of boat noise. There was a significant effect

281 of prey density on the proportion of time the larvae spent swimming ($F_{2, 243} = 3.36$,

282 $p = 0.036$; Fig. 5c), but sound treatment had no effect (boat noise: $F_{1, 243} = 0.32$, $p = 0.57$;

283 boat noise × prey density, $F_{2, 243} = 0.74$, $p = 0.48$). Globally, larvae swam $66 \pm 38.2\%$ of

284 their time. Larvae seemed to spend less time swimming when prey concentration was

285 intermediate, but these differences were not significant according to post-hoc analyses (Fig.

286 5a). However, stomach volume (Fig. 5b) was significantly larger when prey concentration

287 was intermediate (Prey density: $F_{2, 312} = 3.67$, $p = 0.026$). There was no significant prey

288 concentration × boat noise interactions ($F_{2, 312} = 0.488$, $p = 0.61$).

289

290 **Discussion**

291

292 The presence of boat noise had a significant effect on the hunting behaviour of *P.*

293 *americanus* larvae. Larvae exposed to boat noise spent less time hunting and had smaller

294 stomach volumes compared to those with no sound treatment. This suggests that more

295 preys were consumed in the absence of boat noise.

296   In adult fish, noise has been shown to affect predation. Purser and Radford (2011) noted

297 a decrease in foraging performance in three-spined stickleback (*Gasterosteus aculeatus* L.

298 1758) exposed to noise; this decrease resulted from (1) the misidentification of food versus

299   non-food items, as shown by an increased number of attacks on the latter, and (2) fewer

300   successful attacks on food items under noisy conditions.

301   In the present study, the impact of boat noise emission compared to control conditions

302   on larvae was evident, decreasing the number of attacks as well as larval stomach volume,

303   suggesting that many of the observed hunting attempts were not successful. Such

304   differences between the sound treatments cannot be attributed to development since larval

305   fork length was similar among treatments and there were no consistent differences in eye

306   diameter between control and noisy conditions (larvae are visual predators). Indeed, larvae

307   with the less developed eyes (smaller eye diameter) were observed in the "low prey

308   density/absence of boat noise" experimental combination, which showed no difference

309   with other combinations related to boat noise regarding hunting events or stomach volume.

310   Because the distribution of wild winter flounder larvae is not known in the St-Lawrence

311   estuary, we could not work with wild larvae. Our larvae were reared in our wet lab facilities

312   and so were exposed to the wet lab's soundscape in rearing tanks. In the natural

313   environment, the signal-to-noise ratio (SNR) between boat noise and ambient noise was 3.

314   During our experiments, the SNR calculated in aquarium 3 between received boat noise

315   and ambient noise before the experiment is 5.5. Finally, the calculated SNR between the

316   boat noise received in aquarium 3 and the "silent" aquarium 1 at the same time was 4.5.

317   Voellmy et al. (2014) tested the effects of acoustic noise on adult *Gasterosteus aculeatus*

318   and European minnow (*Phoxinus phoxinus*). These authors obtained similar results in that

319   fewer hunting strikes were made by both sympatric species. However, the way in which

320   foraging success was reduced differed: *G. aculeatus* made more foraging errors whereas

321 *P. phoxinus* showed a decrease in foraging efforts. The latter corresponds to the behaviours

322 observed in *P. americanus* larvae, which, as Purser and Radford (2011) suggested, may be

323 related to a shift in attention. Luo et al. (2015) proposed three ways by which noise

324 pollution can affect fish foraging: acoustic masking, reduction of attention, and noise

325 avoidance behaviours. Unfortunately, the present experiment did not allow us to evaluate

326 the presence of acoustic masking or avoidance behaviours. It should be emphasized that in

327 contrast to studies using juveniles or adult fishes, we used larvae that are still early in their

328 development. They do not school, they are very poor swimmers, and drift with currents.

329 Moreover, no interactions among individuals were observed.

330 Since all larvae had their last feeding the night before the experiment and were of similar

331 size, the observed variation in stomach volume supports the conclusion that larvae had a

332 higher feeding success in the absence of boat noise. Licois (2006) confirmed that starving

333 *P. americanus* juveniles (6.4 to 12.2 mm in total length) for 16 h was sufficient to eliminate

334 prey from the digestive system. This supports our assumptions that larva digestive systems

335 were empty at the start of experiments. Furthermore, larvae are transparent at this stage of

336 development, therefore the opaque stomach area observed at the end of the experiment

337 indicated the ingestion of prey and could be easily determined.

338 The differences in stomach size indicate that feeding success was better at the

339 intermediate and high prey densities compared to the low prey density. The results related

340 to swimming budget seem to indicate that less time swimming and large stomachs were

341 associated, which could be related to a higher probability of predator–prey encounters

342 when prey were more abundant. Nevertheless, in the absence of significant comparisons

343   between density treatments, it would be too speculative to provide further explanations.

344   Because prey concentration had no effect on the occurrence of hunting events, it may be

345   argued that the lowest prey concentration was sufficient to fulfill larval needs. Laurence

346   (1977) determined that the critical prey concentration needed for *P. americanus* larvae to

347   meet growth and metabolic energy requirements is around 0.6 copepod nauplii ml^{-1}

348   compared to the mean of 5 rotifers ml^{-1} that was used in our study. Heinle and Flemer

349   (1975) reported a nauplius concentration of *Eurytemora affinis* Poppe 1880 as high as 2.8

350   individuals ml^{-1} in Chesapeake Bay, which was quantified by Laurence (1977) to be more

351   than adequate for growth and survival of *P. americanus* larvae. The lowest prey

352   concentration that was used here was equal to 4.4 rotifers per ml. According to Hansen et

353   al. (1994), nauplii and rotifers have a similar spherical diameter. Therefore, if one considers

354   a size ratio of 1:1 between the two prey types, this further supports the hypothesis that food

355   availability was not a limiting factor during the test. Moreover, since the present

356   experiments were done under laboratory conditions, i.e., with an abundance of prey, clear

357   and well-illuminated waters, and without competitors or predators, we provided optimal

358   environmental conditions for efficient hunting. However, as pointed out by Purser and

359   Radford (2011), the presence of noise in the natural environment is expected to have a

360   greater impact on the species. For example, turbidity would affect hunting success since *P.*

361   *americanus* are visual feeders, and the presence of predators would influence the foraging

362   efficiency of larvae since distraction induced by boat noise may increase the risk of

363   predation. Such hypotheses have been validated by Simpson et al. (2016), who showed that

364   young Ambon damselfish *Pomacentrus amboinensis* exposed to boat noise responded less

365 rapidly to simulated predatory attacks and were six times less likely to be startled by an

366 attack compared with those tested in ambient conditions.

367 Kunc et al. (2014) exposed cuttlefish (*Sepia officinalis* L. 1758), a species that does not

368 rely on acoustic**s** for communication, to a noise playback of an underwater engine noise

369 from a small car ferry. *S. officinalis* showed cross-modal impacts on both visual and tactile

370 sensory modalities (Vermeij 2010; Kunc et al. 2014). Kunc et al. (2014) suggested that

371 noise interference to one sort of sensory channel can affect performance in other sensory

372 channels, thus considering each channel in isolation might lead to misinterpretation of the

373 overall effect of noise pollution in the marine environment. Chan et al. (2010) also

374 confirmed that multi-modal distractions reduce attention to biologically important tasks in

375 the Caribbean hermit crab (*Coenobita clypeatus*). Such observations could be a result of

376 the way sound propagates in water, since underwater acoustic stimuli consist of particle

377 motion as well as sound pressure, both of which can provide information to individuals

378 (Radford et al. 2014). Even though *P. americanus* larvae are visual feeders, the hypothesis

379 of cross-modal impacts cannot be rejected.

380 Using juveniles and adults of different fish species, some studies have strongly

381 suggested the presence of noise habituation. In a field study of juvenile damselfish

382 *Pomacentrus amboinensis*, Holmes et al. (2017) showed that behaviour is altered in

383 response to boat noise, but that the response is no longer present after 20 minutes of

384 exposure, suggesting de-sensitization. In the Australian snapper *Pagrus auratus*, the

385 behavioural response to motorboat sound was different whether observations were done

386 inside or outside protected areas (Mensinger et al. 2018), and when comparing the

387   sensitivity of adult male cichlids (*Cynotilapia zebroides*) in zones characterized by

388   different contrasting levels of motorboat disturbances in Lake Malawi, Harding et al.

389   (2018) also concluded that the response level differed according to the "disturbance

390   history". Habituation has also been suggested in 30 cm European sea bass *Dicentrarchus*

391   *labrax* exposed to repeated impulsive sound (Neo et al. 2018). Could the effect of sound

392   on feeding behaviour be different in winter flounder larvae in coastal environments due to

393   habituation? What is the acclimation and the learning capacity at the larval stage, when

394   energy is devoted to feeding to accumulating enough energy to undertake metamorphosis?

395   Answering these questions will certainly require further investigation. *P. americanus*

396   larvae metamorphose above 6.6 mm length (Vagner et al. 2013; Bélanger et al. 2018).

397   Larvae are poorly developed before metamorphosis, and even though hearing ability has

398   been detected as early as three days post-fertilization in other fish species (Simpson et al.

399   2005), no information is available on the hearing ability of *P. americanus* larvae. Do they

400   respond to sound pressure changes or particle motion (Farkas et al. 2016)? We do not have

401   the answers. However, *P. americanus,* like elasmobranchs and gobies, are among the fish

402   that do not have a swim bladder. This species is therefore considered to possess a pure

403   particle motion detector (Popper and Fay 2011). Future investigations are necessary to

404   completely understand the impacts of noise on the larval stages of marine organisms.

405

406   **Conclusion**

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408   The results of this study demonstrate that boat noise negatively affected the feeding

409   behaviour of *t Ề⬛ṡ̓n̓ḯⱯ▪ĵ ℓ* larvae. There was a significant reduction in hunting attempts

410   in the presence of boat noise, and stomach volume was also reduced. This supports the

411   hypothesis of lower feeding success in the presence of anthropogenic noise, which means

412   that anthropogenic noise may ultimately be another factor susceptible

413   to impact recruitment. Additional analyses focusing on swimming characteristics with the

414   aim of quantifying swimming velocity could help to further enlighten our understanding

415   of the impact of anthropogenic noise on marine vertebrates and more specifically on the

416   larvae of flatfish species.

417

418

419   **Acknowledgments**

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601

602

603 Table 1. Sound levels (dB re $1\mu Pa^2$) measured *in situ* from the vessel noise recorded during

604 the three boat passages, before the experiment in the rearing aquaria, and during the

605 experiment (mean ± SEM for the four trials). There was no sound emission in aquaria 1

606 and 2; sound treatments were performed in aquaria 3 and 4.

607

	100 - 1,000 Hz	1,000 - 10 000 Hz
In situ		
Vessel noise *in situ* (3 passages)	130 ± 1	123 ± 2
Ambient noise (on 24 hours)	86 ± 3	92 ± 2
During rearing		
Aquaria	100 ± 5	87 ± 6
During experiment		
Aquarium 1	108 ± 4	96 ± 5
Aquarium 2	104 ± 1	93 ± 1
Aquarium 3	122 ± 7	114 ± 8
Aquarium 4	119 ± 5	112 ± 8

608

609

610 **Fig. 1** Experimental set-up showing the dimensions (cm) and positions of aquaria

611 containing larvae with the sound source in the water bath under the aquarium.

612

613 **Fig 2** *Pseudopleuronectes americanus larva* at the stage of development used in the present

614 study.

615

616 **Fig. 3** Spectrum (dB re $1\mu Pa^2\ Hz^{-1}$) of recorded sounds: boat noise recorded in situ (black

617 line), in the two aquaria with sound treatment (blue lines), and in the two aquaria with no

618 sound treatment (green lines). The gray shaded area corresponds to variations of natural

619 ambient noise estimated from Wenz's formula for different wind (wind speeds from 0 to

620 10 m s^{-1}) and traffic (traffic density from 1 to 7) conditions.

621

622 **Fig. 4** Fork length (a) and eye diameter (b) of *Pseudopleuronectes americanus* larvae used

623 in the different treatments (prey density: low, medium, high; boat noise: absent, present).

624 Different letters indicate significantly different means among treatments ($P < 0.05$). Mean

625 ± S.D.

626

627 **Fig. 5** Mean number of hunts per 12 minutes per *Pseudopleuronectes americanus* larva (a),

628 stomach volume (b) and percentage of time *Pseudopleuronectes americanus* larvae spent

629 swimming stomach volume (c) in absence or presence of boat noise and fed with three

630 different prey densities. Different letters indicate significantly different means among prey

631   densities. Significant sound effects are indicated on panels. No significant interactions

632   between factors were present (see the Results section). Mean ± S.D.
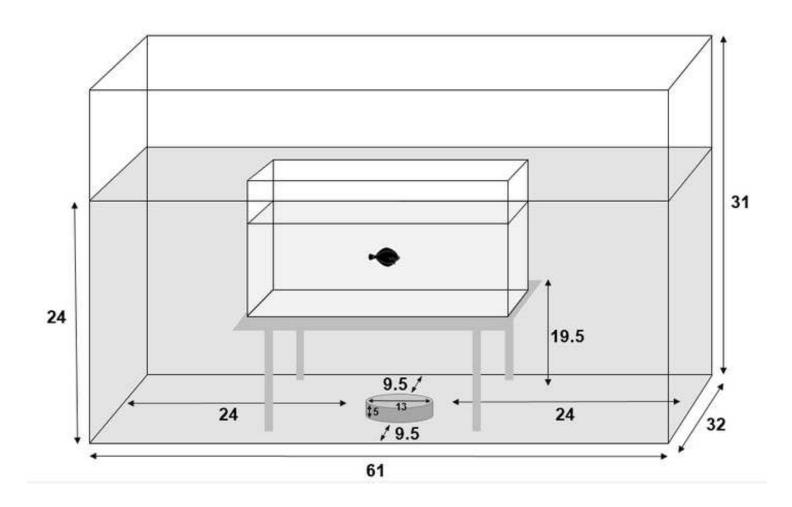
Figure 1

Figure 2

Figure 4

Figure 5