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1 Anthropogenic boat noise reduces feeding success in winter flounder larvae

2 (*Pseudopleuronectes americanus*)

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8 **18 Abstract**
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13 **20** The aim of this study was to explore an emerging discipline addressing the impact of
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15 **21** anthropogenic noise on larval stages of marine organisms. We assessed the influence of
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17 **22** boat noise on the feeding behaviour of the pelagic larvae of winter flounder
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19 **23** (*Pseudopleuronectes americanus*, Walbaum 1792). The hypothesis was that boat noise
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21 **24** influences the feeding behaviour of *P. americanus* flounder larvae independently of prey
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23 **25** density. Aquaria containing *P. americanus* larvae were placed in water baths in which boat
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25 **26** noise was diffused for the “noise” treatment and compared to control aquaria with no sound
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27 **27** emissions. Larvae were filmed using cameras placed above the aquaria and their behaviour
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29 **28** was recorded. Larvae exposed to anthropogenic noise displayed significantly fewer
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31 **29** hunting events than controls, and their stomach volumes were significantly smaller. This
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33 **30** noise effect was the same at all prey densities used, suggesting that larval feeding behaviour
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35 **31** is negatively impaired by anthropogenic noise.
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43 **32**

44 **33 Keywords:** Feeding behaviour, winter flounder, larvae, anthropogenic noise, vessel noise
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37 **Introduction**

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39 Ambient underwater sound in the aquatic environment is used by marine organisms for
40 orientation, communication among individuals, predator avoidance, and prey detection,
41 and it can also be used as a larval settlement cue in various species, especially among those
42 residing in coastal areas (Montgomery et al. 2006; Götz et al. 2009; Slabbekoorn et al.
43 2010; Stanley et al. 2010). However, anthropogenic underwater noise resulting from
44 increasing maritime navigation, offshore wind and tidal turbines, mariculture facilities,
45 piling installations, and seismic surveys for oil and gas exploration can have detrimental
46 impacts on many marine organisms (Southall et al. 2007; Popper and Hastings 2009;
47 Slabbekoorn et al., 2010; Popper et al. 2014; Kunc et al. 2016; Cox et al. 2018). These
48 negative effects will likely increase since anthropogenic noise levels are rising with
49 increasing human activities (Williams et al. 2015).

50 Studies on the effects of noise on foraging have been done with different life stages and
51 different fish species, but studying larval stages is of special interest (high sensitivity to
52 abiotic factors; the importance of larval survival in population recruitment) and poses
53 specific challenges. Larval marine organisms have been shown to be sensitive to aquatic
54 noise. Invertebrate larvae can detect and respond to aquatic noise (Simpson et al. 2011).
55 Using the aquatic larval stage of damselfly, a freshwater invertebrate feeding on *Daphnia*
56 during this aquatic phase, Villabulos-Jimenez et al. (2017) obtained results suggesting that
57 underwater noise may decrease feeding. Anthropogenic noise may cause malformations or
58 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

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8 81 determine how motorized boat noise combined with prey density would affect feeding
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10 82 success during this critical stage. We hypothesized that anthropogenic vessel noise would
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12 83 impair feeding success during feeding on live prey and that this effect would be more
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15 84 pronounced with lower prey availability. Winter flounder is a dominant benthic species in
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17 85 the St. Lawrence estuary (Bigelow and Schroeder 1953; Pereira et al. 1999) and is present
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19 86 in areas where a local rise in ambient noise levels has been noted (Gervaise et al. 2012).
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21 87 Winter flounder sport fishing is economically important in North America (Fairchild 2008;
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23 88 Fishwatch 2019), including Québec (DFO, 2012). However, over the last few decades,
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25 89 most populations including that of the St. Lawrence estuary have encountered marked
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27 90 decreases for reasons that remain to be identified. Conservation issues thus require research
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29 91 efforts on all factors that may affect recruitment. Like many marine vertebrates, *P.*
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31 92 *americanus* has a complex life cycle. Upon hatching, larvae are pelagic until they undergo
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33 93 metamorphosis to the juvenile stage (Pereira et al. 1999). During this pelagic larval phase,
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35 94 feeding success is vital for growth and for accumulating enough energy for successful
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37 95 metamorphosis. Larvae are visual feeders and spend most of the critical periods of their
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39 96 development in shallow coastal areas, which are especially vulnerable to boat noise. Thus,
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41 97 this species is likely a good model to assess the impact of anthropogenic noise on marine
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43 98 species that use coastal areas during critical stages of their life history.
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100 **Material and methods**

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102 Larval production

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10 104 Mature *P. americanus* females were captured in the Baie des Chaleurs offshore of
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12 105 Bonaventure (QC, Canada) during the breeding season and brought to the Station aquicole
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14 106 de l'ISMER (Rimouski, QC, Canada; 48° 31' N; 68° 28' W). Fertile males were available
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16 107 at the wet lab facility. Egg fertilization and incubation were done according to the method
17
18 108 described by Vagner et al. (2013). Upon hatching, larvae were reared according to the
19
20 109 standard procedures used in our laboratory (Vagner et al. 2013; Martinez-Silva et al. 2018).
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22 110 Briefly, larvae were transferred into 55 L cylindro-conical fiberglass tanks and reared in
23
24 111 green water at 10°C with a 12h:12h L:D photoperiod. Larval density was set to 1 larva
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26 112 ml⁻¹, gentle aeration was used to create upwelling, and a constant flow of filtered (1 µm)
27
28 113 seawater was provided. In larval rearing tanks, the water supply was stopped each day for
29
30 114 12 h while a green water preparation (*Nannochloropsis oculata* at 1.6×10⁶ cells L⁻¹) was
31
32 115 added to each tank. At the end of the day, water circulation was restored, allowing complete
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34 116 renewal of the tank water overnight. Larvae were routinely co-fed with rotifers, *Brachionus*
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36 117 *plicatilis*, and a commercial diet (GEMMA Wean 0.3, SKRETTING, France) was added
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38 118 to their regime once they reached 5.5 mm (Ben Khemis et al. 2003; Vagner et al. 2013;
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40 119 Martinez-Silva et al. 2018).
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51 121 Underwater sound
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56 123 As described in Jolivet et al. (2016), the vessel noise emitted during the experiments was
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58 124 initially recorded at a mussel aquaculture site at St. Peter's Bay on Prince Edward Island
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8 125 (Canada; 46° 25.963' N; 62° 39.914' W). The calibrated hydrophone (High Tech, Inc.,
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10 126 Mississippi, USA, HTI-99-HF: sensitivity -169.7 dB re 1 V/ μ Pa; frequency range 2 Hz to
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12 127 125 kHz flat response) was placed 25 cm from the bottom, near the anchor of the mussel
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14 128 line, and connected to an underwater acoustic recorder (RTSYS-Marine Technologies,
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16 129 France, EA-SDA14, 156 kHz, 24-bit resolution). The farmer's boat (11 m in length; D &
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18 130 H Boatbuilding hull with diesel motor, Cummins 300 hp C series) passed three times above
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20 131 the recording hydrophone during calm natural conditions characterized by a wave height
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22 132 of 0.2 m and wind speed of 3.8 m s⁻¹ (<http://climat.meteo.gc.ca/>). From the recording, a
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24 133 sequence lasting 30 s that corresponded to the maximum vessel noise sound intensity was
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26 134 selected using customized codes written in MATLAB (The MathWorks, Inc.) software.
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35 136 Experimental design

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39 138 For the experiments, four 40 L aquaria were used, each containing 30 L of water. A
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41 139 rectangular 2 L glass container was placed on a platform 19.5 cm from the aquarium's
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43 140 bottom so that the upper rim of the aquarium was 1 cm above the water's surface (Fig. 1).
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45 141 Water temperature in the aquaria was constant and monitored by probes (Onset Hobo
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47 142 Water Temp Pro V2 Data logger U22-001); the average temperature was $12.1 \pm 0.4^\circ\text{C}$ with
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49 143 a mean increase of $1.2 \pm 0.2^\circ\text{C}$ between the start and the end of experiments (2 h). Low-
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51 144 intensity lights were installed above each aquarium to reproduce a light intensity present
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53 145 in the production room (around 400 lux).
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8 146 Twenty experimental larvae were randomly sampled in the water column of the rearing
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10 147 tank prior to the morning feeding, which occurred when the lights were turned on in the
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12 148 production room. Larvae were transferred into the 2 L containers (1.8 L of water) to
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15 149 acclimate for 1 h prior to the start of the experiment and were not fed during the acclimation
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17 150 to ensure feeding during the experiment. No boat noise was played during the acclimation
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20 151 period. A digital camera (GoPro, Hero+ LCD CHDHB-401) fitted with a macro filter
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22 152 (Polar Pro, macro filter 15-06746) was installed above each aquarium to record larval
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25 153 behaviour for 2 h following the acclimation period (Fig. 1).
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27 154 For each experimental trial, larval behaviour was monitored simultaneously in the
28
29 155 presence or absence of vessel noise; there were two aquaria per treatment and four
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31 156 experimental trials were carried out. Individual larval behaviour was then monitored for 20
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33 157 larvae \times two aquaria \times four trials, for a total of 160 larvae in the presence of boat noise and
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35 158 160 larvae with no noise. Larvae could not see the sound source because the bottom of the
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37 159 aquarium was opaque to facilitate their tracking. In the experimental tanks, larvae were
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39 160 only offered rotifers, with no commercial diet. Prey density was low in the first trial ($5 \pm$
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41 161 0.6 rotifers ml^{-1}), medium in the second and third trials (11 ± 2.0 rotifers ml^{-1}), and high in
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43 162 the fourth trial (14 ± 0.8 rotifers ml^{-1}). The low and medium densities correspond to prey
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45 163 densities previously used with success along with co-feeding with commercial diet (Vagner
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47 164 et al. 2013; Martinez-Silva et al. 2018).
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167 Sound emissions

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10 169 Waterproof speakers (AQUA 30, DNH, 8 ohms, 80–20,000 Hz), plugged to an amplifier
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12 170 (Plug & Play 12 W) and connected to a PC, were installed in two of the four aquaria to
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15 171 continuously replay the vessel noise. The sound emission under experimental conditions
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17 172 was calibrated using a calibrated hydrophone (HTI-96 MIN; High Tech, Inc.) associated
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19 173 with a digital recorder (Song Meter SM2+; Wildlife Acoustics). The source was placed in
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21 174 the center of the aquarium just below the glass container. The multiple reflections off the
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23 175 sides of the aquaria produced homogeneous sound conditions (S.E.: ± 1.5 dB) in the glass
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25 176 container, which was confirmed by sound measurements performed in each corner and the
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27 177 center prior the experiment. A correction function was calculated from 30 s recordings of
28
29 178 calibrated sound done in each corner and the center, and this was applied to the vessel noise
30
31 179 to replicate the *in situ* spectrum of the vessel noise as nearly as possible. By varying the
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33 180 gain of the amplifier, the intensity was adjusted to match natural conditions (sound level
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35 181 [SL]: 130 dB re $1\mu\text{Pa}^2$ between 100 and 10,000 Hz). Two recordings were also made in
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37 182 adjacent basins to check the “silent” conditions (located 1.5 m away).
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46 184 Rotifer production and counts

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51 186 Rotifers were reared in an 18 L tank and fed with Selco® S.parkle (INVE Aquaculture
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53 187 Ltd., Thailand) four times a day. The culture concentration was estimated every morning,
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55 188 and the volume of culture needed to reach a given prey concentration in each experimental
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57 189 aquarium was calculated.
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8 190 After the 1 h acclimation period, the video recording was started, rotifers (prey) were
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10 191 added to each experimental aquarium, and seawater was added to top-up each aquarium to
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12 192 2 L. At the end of the experiments, subsamples of rotifer cultures and seawater from each
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15 193 aquarium were filtered through a 10 μm sieve and preserved in 95% ethanol for prey
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18 194 concentration assessment. Rotifer concentration was estimated both before and after the
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20 195 experiments by diluting the filtered rotifer samples in 80 ml of ethanol and counting rotifers
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22 196 in 3 ml aliquots under a binocular microscope (three aliquots per sample).
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27 198 Behaviour analysis
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32 200 The videos were analyzed using Noldus Observer XT 9.0 software (Noldus Information
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34 201 Technology Inc., Leesburg, VA, USA). A delimited field covering 95% of the aquarium's
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37 202 bottom but avoiding sides and corners, where visibility was impaired, was used to analyze
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39 203 the videos. Only larvae found within this delimited field were considered. A period of 12
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42 204 minutes, always from minute 12 to 24 of the 2 h video for standardization purposes, was
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44 205 analyzed for occurrence frequencies and duration of each behavioural trait (in seconds) for
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46 206 each replicate and for each larva present in the observation field, giving a total of 132 larvae
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49 207 in the absence and 117 in the presence of boat noise. The various observed and recorded
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52 208 behaviours were set to be mutually exclusive state events except for hunting, which was
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54 209 considered as a point event. The following behaviours were recorded: (1) Hunting: larva
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56 210 moves rapidly with a wiggling movement; (2) Swimming: larva moves around actively in
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59 211 the water column; (3) Resting: larva undertakes no active movement but rather stays
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212 motionless on the bottom or floats passively; (4) Out: larva swims out of the observation
213 field.

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215 Morphometric measurements

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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).

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228 Statistical analysis

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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 ($\alpha = 0.05$). 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 \times 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

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252 The replayed vessel noise in the two aquaria was homogenous, with 129 and 127
253 dB re $1\mu\text{Pa}^2$ 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

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8 256 levels in the aquaria with no sound treatment were slightly higher than the conditions
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10 257 before the experiments (Table 1) but remained consistent with natural conditions as defined
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13 258 by Wenz's formula (Wenz 1962) (Fig. 3).

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18 260 Predation analysis

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22 262 Even though we used larvae of the same age, fork length and eye diameter differed slightly
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25 263 among treatments (Fig. 4a, b). There was a significant boat noise \times prey density effect
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27 264 ($F_{2,312} = 3.65, p = 0.027$) on larval fork length. However, the *a posteriori* test failed to find
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30 265 a significant difference among treatments, and the overall mean larval fork length (\pm S.D.)
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32 266 was 4.77 ± 0.515 mm. A significant boat noise \times prey density effect ($F_{2,312} = 4.09,$
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35 267 $p = 0.018$) was also observed for eye diameter (Fig. 4b): larvae with the smallest eyes were
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37 268 from the low prey density/absence of boat noise combination. As stated above, nested
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40 269 ANOVA failed to indicate any differences between replicate aquaria for each experiment,
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42 270 and this was also the case for fork length and eye diameter.

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44 271 The variation in prey concentration had no effect on the hunting behaviour ($F_{2, 243} =$
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47 272 $1.28, p = 0.28$) while hunting attempts were 34% less frequent in the presence of sound
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50 273 compared to the treatment when sound treatment was absent ($F_{1, 243} = 6.75, p = 0.010$; Fig.
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52 274 5a). There was no interaction between prey concentration and sound treatment ($F_{2, 243} =$
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54
55 275 $1.71, p = 0.18$) on the number of hunts. Stomach volume measurements (Fig. 5b) showed
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57 276 that stomachs were 13% smaller in the presence of boat noise ($F_{1, 312} = 4.29, p = 0.039$),
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8 277 and this effect was independent of prey density (boat noise \times prey density, $F_{2, 312} = 0.488$,
9 278 $p = 0.614$).

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12 279 Prey concentration significantly influenced some of the measured variables, but without
13 280 interaction effects with the presence or absence of boat noise. There was a significant effect
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15 281 of prey density on the proportion of time the larvae spent swimming ($F_{2, 243} = 3.36$,
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17 282 $p = 0.036$; Fig. 5c), but sound treatment had no effect (boat noise: $F_{1, 243} = 0.32$, $p = 0.57$;
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19 283 boat noise \times prey density, $F_{2, 243} = 0.74$, $p = 0.48$). Globally, larvae swam $66 \pm 38.2\%$ of
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21 284 their time. Larvae seemed to spend less time swimming when prey concentration was
22
23 285 intermediate, but these differences were not significant according to post-hoc analyses (Fig.
24
25 286 5a). However, stomach volume (Fig. 5b) was significantly larger when prey concentration
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27 287 was intermediate (Prey density: $F_{2, 312} = 3.67$, $p = 0.026$). There was no significant prey
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29 288 concentration \times boat noise interactions ($F_{2, 312} = 0.488$, $p = 0.61$).

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33 290 **Discussion**

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35 292 The presence of boat noise had a significant effect on the hunting behaviour of *P.*
36 293 *americanus* larvae. Larvae exposed to boat noise spent less time hunting and had smaller
37 294 stomach volumes compared to those with no sound treatment. This suggests that more
38 295 preys were consumed in the absence of boat noise.

39 296 In adult fish, noise has been shown to affect predation. Purser and Radford (2011) noted
40 297 a decrease in foraging performance in three-spined stickleback (*Gasterosteus aculeatus* L.
41 298 1758) exposed to noise; this decrease resulted from (1) the misidentification of food versus

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8 299 non-food items, as shown by an increased number of attacks on the latter, and (2) fewer
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10 300 successful attacks on food items under noisy conditions.

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12 301 In the present study, the impact of boat noise emission compared to control conditions
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14 302 on larvae was evident, decreasing the number of attacks as well as larval stomach volume,
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16 303 suggesting that many of the observed hunting attempts were not successful. Such
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18 304 differences between the sound treatments cannot be attributed to development since larval
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20 305 fork length was similar among treatments and there were no consistent differences in eye
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22 306 diameter between control and noisy conditions (larvae are visual predators). Indeed, larvae
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24 307 with the less developed eyes (smaller eye diameter) were observed in the “low prey
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26 308 density/absence of boat noise” experimental combination, which showed no difference
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28 309 with other combinations related to boat noise regarding hunting events or stomach volume.
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33 310 Because the distribution of wild winter flounder larvae is not known in the St-Lawrence
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35 311 estuary, we could not work with wild larvae. Our larvae were reared in our wet lab facilities
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37 312 and so were exposed to the wet lab’s soundscape in rearing tanks. In the natural
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39 313 environment, the signal-to-noise ratio (SNR) between boat noise and ambient noise was 3.
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41 314 During our experiments, the SNR calculated in aquarium 3 between received boat noise
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43 315 and ambient noise before the experiment is 5.5. Finally, the calculated SNR between the
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45 316 boat noise received in aquarium 3 and the “silent” aquarium 1 at the same time was 4.5.
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51 317 Voellmy et al. (2014) tested the effects of acoustic noise on adult *Gasterosteus aculeatus*
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53 318 and European minnow (*Phoxinus phoxinus*). These authors obtained similar results in that
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55 319 fewer hunting strikes were made by both sympatric species. However, the way in which
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57 320 foraging success was reduced differed: *G. aculeatus* made more foraging errors whereas
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8 321 *P. phoxinus* showed a decrease in foraging efforts. The latter corresponds to the behaviours
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10 322 observed in *P. americanus* larvae, which, as Purser and Radford (2011) suggested, may be
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12 323 related to a shift in attention. Luo et al. (2015) proposed three ways by which noise
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15 324 pollution can affect fish foraging: acoustic masking, reduction of attention, and noise
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18 325 avoidance behaviours. Unfortunately, the present experiment did not allow us to evaluate
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20 326 the presence of acoustic masking or avoidance behaviours. It should be emphasized that in
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22 327 contrast to studies using juveniles or adult fishes, we used larvae that are still early in their
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25 328 development. They do not school, they are very poor swimmers, and drift with currents.
26
27 329 Moreover, no interactions among individuals were observed.

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29 330 Since all larvae had their last feeding the night before the experiment and were of similar
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32 331 size, the observed variation in stomach volume supports the conclusion that larvae had a
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35 332 higher feeding success in the absence of boat noise. Licois (2006) confirmed that starving
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37 333 *P. americanus* juveniles (6.4 to 12.2 mm in total length) for 16 h was sufficient to eliminate
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40 334 prey from the digestive system. This supports our assumptions that larva digestive systems
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42 335 were empty at the start of experiments. Furthermore, larvae are transparent at this stage of
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44 336 development, therefore the opaque stomach area observed at the end of the experiment
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47 337 indicated the ingestion of prey and could be easily determined.

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49 338 The differences in stomach size indicate that feeding success was better at the
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52 339 intermediate and high prey densities compared to the low prey density. The results related
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54 340 to swimming budget seem to indicate that less time swimming and large stomachs were
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57 341 associated, which could be related to a higher probability of predator–prey encounters
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59 342 when prey were more abundant. Nevertheless, in the absence of significant comparisons
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8 343 between density treatments, it would be too speculative to provide further explanations.
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10 344 Because prey concentration had no effect on the occurrence of hunting events, it may be
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12 345 argued that the lowest prey concentration was sufficient to fulfill larval needs. Laurence
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15 346 (1977) determined that the critical prey concentration needed for *P. americanus* larvae to
16
17 347 meet growth and metabolic energy requirements is around 0.6 copepod nauplii ml⁻¹
18
19 348 compared to the mean of 5 rotifers ml⁻¹ that was used in our study. Heinle and Flemer
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21 349 (1975) reported a nauplius concentration of *Eurytemora affinis* Poppe 1880 as high as 2.8
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23 350 individuals ml⁻¹ in Chesapeake Bay, which was quantified by Laurence (1977) to be more
24
25 351 than adequate for growth and survival of *P. americanus* larvae. The lowest prey
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27 352 concentration that was used here was equal to 4.4 rotifers per ml. According to Hansen et
28
29 353 al. (1994), nauplii and rotifers have a similar spherical diameter. Therefore, if one considers
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31 354 a size ratio of 1:1 between the two prey types, this further supports the hypothesis that food
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33 355 availability was not a limiting factor during the test. Moreover, since the present
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35 356 experiments were done under laboratory conditions, i.e., with an abundance of prey, clear
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37 357 and well-illuminated waters, and without competitors or predators, we provided optimal
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39 358 environmental conditions for efficient hunting. However, as pointed out by Purser and
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41 359 Radford (2011), the presence of noise in the natural environment is expected to have a
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43 360 greater impact on the species. For example, turbidity would affect hunting success since *P.*
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45 361 *americanus* are visual feeders, and the presence of predators would influence the foraging
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47 362 efficiency of larvae since distraction induced by boat noise may increase the risk of
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49 363 predation. Such hypotheses have been validated by Simpson et al. (2016), who showed that
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51 364 young Ambon damselfish *Pomacentrus amboinensis* exposed to boat noise responded less
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8 365 rapidly to simulated predatory attacks and were six times less likely to be startled by an
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10 366 attack compared with those tested in ambient conditions.

11
12 367 Kunc et al. (2014) exposed cuttlefish (*Sepia officinalis* L. 1758), a species that does not
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14
15 368 rely on acoustics for communication, to a noise playback of an underwater engine noise
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18 369 from a small car ferry. *S. officinalis* showed cross-modal impacts on both visual and tactile
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20 370 sensory modalities (Vermeij 2010; Kunc et al. 2014). Kunc et al. (2014) suggested that
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22 371 noise interference to one sort of sensory channel can affect performance in other sensory
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25 372 channels, thus considering each channel in isolation might lead to misinterpretation of the
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27
28 373 overall effect of noise pollution in the marine environment. Chan et al. (2010) also
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30 374 confirmed that multi-modal distractions reduce attention to biologically important tasks in
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32 375 the Caribbean hermit crab (*Coenobita clypeatus*). Such observations could be a result of
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35 376 the way sound propagates in water, since underwater acoustic stimuli consist of particle
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38 377 motion as well as sound pressure, both of which can provide information to individuals
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40 378 (Radford et al. 2014). Even though *P. americanus* larvae are visual feeders, the hypothesis
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42 379 of cross-modal impacts cannot be rejected.

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44 380 Using juveniles and adults of different fish species, some studies have strongly
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47 381 suggested the presence of noise habituation. In a field study of juvenile damselfish
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49 382 *Pomacentrus amboinensis*, Holmes et al. (2017) showed that behaviour is altered in
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52 383 response to boat noise, but that the response is no longer present after 20 minutes of
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54 384 exposure, suggesting de-sensitization. In the Australian snapper *Pagrus auratus*, the
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57 385 behavioural response to motorboat sound was different whether observations were done
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59 386 inside or outside protected areas (Mensingher et al. 2018), and when comparing the
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8 387 sensitivity of adult male cichlids (*Cynotilapia zebroides*) in zones characterized by
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10 388 different contrasting levels of motorboat disturbances in Lake Malawi, Harding et al.
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12 389 (2018) also concluded that the response level differed according to the “disturbance
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15 390 history”. Habituation has also been suggested in 30 cm European sea bass *Dicentrarchus*
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17 391 *labrax* exposed to repeated impulsive sound (Neo et al. 2018). Could the effect of sound
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19 392 on feeding behaviour be different in winter flounder larvae in coastal environments due to
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21 393 habituation? What is the acclimation and the learning capacity at the larval stage, when
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23 394 energy is devoted to feeding to accumulating enough energy to undertake metamorphosis?
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26 395 Answering these questions will certainly require further investigation. *P. americanus*
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28 396 larvae metamorphose above 6.6 mm length (Vagner et al. 2013; Bélanger et al. 2018).
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30 397 Larvae are poorly developed before metamorphosis, and even though hearing ability has
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32 398 been detected as early as three days post-fertilization in other fish species (Simpson et al.
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34 399 2005), no information is available on the hearing ability of *P. americanus* larvae. Do they
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36 400 respond to sound pressure changes or particle motion (Farkas et al. 2016)? We do not have
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38 401 the answers. However, *P. americanus*, like elasmobranchs and gobies, are among the fish
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40 402 that do not have a swim bladder. This species is therefore considered to possess a pure
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42 403 particle motion detector (Popper and Fay 2011). Future investigations are necessary to
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44 404 completely understand the impacts of noise on the larval stages of marine organisms.
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406 **Conclusion**

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8 408 The results of this study demonstrate that boat noise negatively affected the feeding
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10 409 behaviour of *t. E. s. l.* larvae. There was a significant reduction in hunting attempts
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12 410 in the presence of boat noise, and stomach volume was also reduced. This supports the
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14 411 hypothesis of lower feeding success in the presence of anthropogenic noise, which means
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16 412 that anthropogenic noise may ultimately be another factor susceptible
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18 413 to impact recruitment. Additional analyses focusing on swimming characteristics with the
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20 414 aim of quantifying swimming velocity could help to further enlighten our understanding
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22 415 of the impact of anthropogenic noise on marine vertebrates and more specifically on the
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24 416 larvae of flatfish species.
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43
44 425 and experiment setup. All experiments were conducted at the Station aquicole de Pointe-
45
46 426 au-Père (ISMER/UQAR, 48°27'N;68°32'W, QC, Canada), and all breeders and larval
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48 427 manipulations were done according to the Canadian Council of Animal Protection
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50 428 recommendations and protocols approved by the University's Animal Care Committee.
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603 Table 1. Sound levels (dB re 1 μ Pa²) 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 \pm 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
<i>In situ</i>		
Vessel noise <i>in situ</i> (3 passages)	130 \pm 1	123 \pm 2
Ambient noise (on 24 hours)	86 \pm 3	92 \pm 2
During rearing		
Aquaria	100 \pm 5	87 \pm 6
During experiment		
Aquarium 1	108 \pm 4	96 \pm 5
Aquarium 2	104 \pm 1	93 \pm 1
Aquarium 3	122 \pm 7	114 \pm 8
Aquarium 4	119 \pm 5	112 \pm 8

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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 $1\mu\text{Pa}^2 \text{Hz}^{-1}$) 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 \pm 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

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8 631 densities. Significant sound effects are indicated on panels. No significant interactions

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10 632 between factors were present (see the Results section). Mean \pm S.D.
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Figure 1

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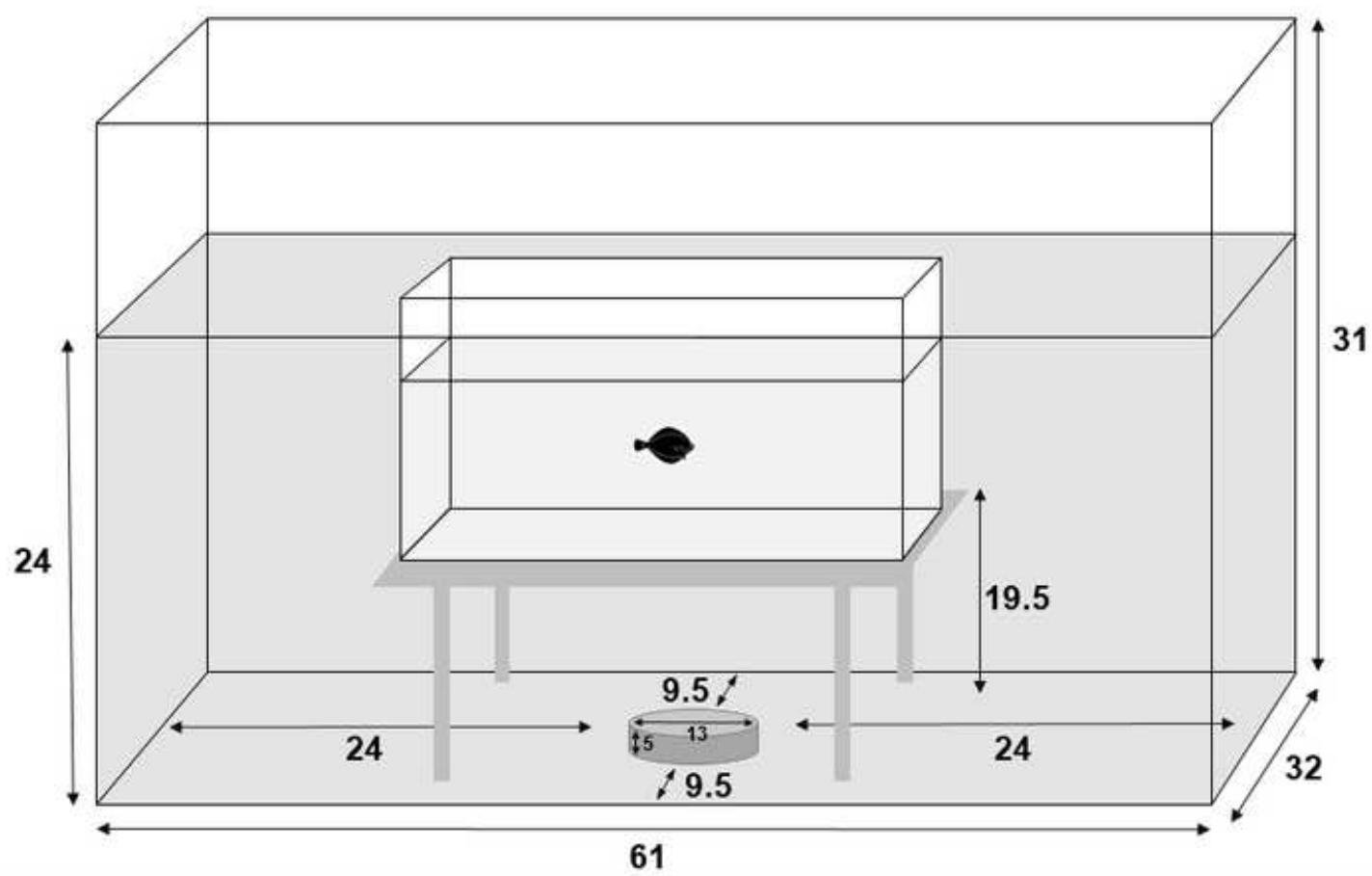


Figure 2

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