1	Territoriality, courtship and 'prop' use in the tompot blenny
2	Parablennius gattorugine from a long-term photographic record.
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24	Running headline: tompot blenny territoriality and courtship
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26 Abstract

27 The behaviour of the tompot blenny Parablennius gattorugine was studied from a 28 long-term underwater photographic record on a Devon (UK) reef. Repeated 29 observations of individually identifiable fish during 69 dives revealed that male 30 blennies may guard eggs in a particular crevice over subsequent breeding seasons and 31 reside in the same location for up to four years. Resident males were observed wiping 32 eggs with greatly expanded anal glands and defending their territory against rivals, 33 sustaining and then recovering from resultant fighting injuries. Adult-type fighting 34 was also recorded between juveniles within a few weeks of settling from the 35 planktonic larval stage. Our long-term record reveals the complexities of P. 36 gattorugine courtship behaviour with further observations in Dorset (UK) showing a 37 male manipulating an empty mollusc shell in an apparent display of prowess while 38 being closely observed by two prospective female mates. Photographs of these 39 extraordinary behaviours in this charismatic benthic species are provided.

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41 Keywords: benthic ecology; competition; observations; reproductive behaviours

43 Introduction

Shallow-water species make excellent subjects in which to observe the complex
behaviours associated with territoriality and courtship in marine teleost fishes.
However, monitoring individuals and their interactions in the wild over long periods,
even in shallow water, is difficult and such data are often elusive, despite the advent
of new biologging technologies.

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50 The tompot blenny Parablennius gattorugine Brunnich 1768 is a common fish of 51 shallow, sub-littoral rocky habitats (Maitland & Herdson, 2009) and is widely 52 distributed in Northern Europe (Almada et al, 2001). Although visually appealing and 53 charismatic, its biology and behaviour, particularly its reproductive strategy, has been 54 little studied (Dunne & Byrne, 1979; Faria et al, 2010). This species is a fractional 55 spawner, (i.e. multiple batches per season, Dunne and Byrne, 1979) with the female 56 laying demersal eggs in the late spring and early summer (Wheeler, 1969; Maitland & 57 Herdson, 2009). The male guards eggs laid by several females in its resident rocky 58 crevice (Zander, 1986), in a similar way to other blenny species (Westernhagen, 59 1983) and many shallow-water marine teleosts in general (Cody, 1993). Zander 60 (1986) notes "mouthing fighting" attacks by rival male P. gattorugine but there appears to be no information on the courtship behaviour of this species in the 61 62 scientific literature, nor on whether home ranges are retained by males between 63 breeding seasons (Kay & Dipper, 2009).

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The reproduction of closely related species *Parablennius tentacularis* (the tentacled
blenny) and *Parablennius parvicornis* (the Azorean rock-pool blenny) suggest some
consistent patterns throughout the combtooth blenny family (Giacomello & Rasotto,

68 2005; Oliveira *et al*, 2009 respectively), with descriptions of the mating system of 69 these fishes as resource defence polygyny. Almada *et al* (1995) discuss the courtship 70 behaviour of male blennies at their residences and note that sex-role reversal in 71 courtship can occur when the operational sex ratio is biased towards females. 72 Reproduction in blennies is complex and the courtship behaviour of different species 73 (reviewed by Neat and Lengkeek, 2009) is therefore worthy of further attention, 74 particularly in a species as widespread but little-studied as *P. gattorugine*.

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This study demonstrates how underwater observation and photography can reveal intriguing information about reproductive behaviour, particularly when carried out in the same location over extended periods so that the life events of individual animals are recorded. Discussion of blenny behaviour also includes a very unusual record of prop use as a show of male prowess during courtship. Observed examples of tool use in fishes have so far been limited to food capture and access, nest building and egg care in a small number of taxa (see Brown, 2012 for review).

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84 Materials and methods

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1. Devon UK, Wembury Bay, 50.3163 °N, -4.1153 °W: Observations of *P. gattorugine* were made during 69 dives between 2011 and 2014 in a small area of algae-covered rocky reef in which there are several horizontal crevices. The reef is approximately 2 metres tall and water depth at the base of the reef varies between 3 and 8 metres, depending on tidal state. Observations were generally made around high water, as this gave easier swimming access from the shore (approximately 400 metres).

^{86 &}lt;u>Sites</u>

away) and better water visibility than at low water. Observations were made atdifferent times throughout the day due to tidal influence.

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96 2. Dorset UK, Swanage, 50.6089 °N, -1.9491 °W: Observations of *P. gattorugine*97 were made during one dive on 25 September 2011, in an area of seabed with stones
98 and small boulders on a sandy base beneath the main Swanage pier.

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100 Data collection

Dives were made with open-circuit Self Contained Underwater Breathing Apparatus
(SCUBA). Photographs were taken with a Nikon digital SLR camera and Sigma 1770mm zoom lens in a waterproof housing. Lighting was provided by two small
electronic flashguns mounted on flexible arms attached to the camera housing.

Areas where blennies were active were approached very slowly to minimise disturbance. Observations were made and photographs taken while resting gently (kneeling or standing) with near-neutral buoyancy on the sandy seabed adjacent to the reef or rocks where the blennies resided. The camera was positioned between 25 and 70 cm away from the blennies depending on visibility. Blennies did not appear disturbed by the presence of the camera, particularly when there was interaction between individuals.

Photographs were examined in order to identify individual *P. gattorugine*, from the patterns of markings on their skin. Markings on the head were used, as this is the easiest part of a blenny to photograph when it is residing in a crevice. The pattern of markings on the side of the head beneath the eyes was found to be particularly easy to recognise and could be used to identify individual blennies with a high degree of certainty (Fig. 1).

118 The gender of generally larger and darker P. gattorugine, including those 119 occupying crevices, was confirmed as male by the observation of an anal/bulb gland 120 on each of the front two fin-rays of the anal fin (Zander, 1975; Giacomello & Rasotto, 121 2005). Anal glands were enlarged and particularly obvious when the males were 122 guarding eggs (see Fig. 2). Female blennies were generally paler and slightly smaller, 123 and did not have visible anal glands. They often had a prominent pale patch mark 124 beneath the eye (e.g. Fig. 6a, b). In addition to identifying individual blennies, 125 photographs were used to record behaviour and, for the Dorset observations, to track 126 the relative positions of blennies and the mollusc shell manipulated by the male. The 127 mollusc shell was examined and measured after the blennies had left the area, and 128 movements were measured relative to the length of the shell (80 mm) on photographs.

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130 **<u>Results</u>**

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132 <u>Male occupancy of residences</u>

133 Male *P. gattorugine* (n = 5) demonstrated long-term occupancy of three particular 134 crevice locations on the Devon reef between 2011 and 2014 (Fig. 3). Crevice 135 locations A and B were adjacent parts of the same horizontal fissure, approximately 136 80 cm apart, while crevice location C was 120 cm along the reef from B and 40 cm 137 higher up the reef. Individual males typically occupied the same location over at least 138 two years. Where this extended over three or four years, there were intervening 139 changes in occupancy during that time.

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141 <u>Territoriality of P. gattorugine males</u>

142 Aggression was observed between rival male P. gattorugine and appeared to be in 143 competition for occupancy of crevices. Damage around a blenny's mouth occurred on 144 at least two occasions, before subsequently healing (Fig. 4). Mouth and fin damage, 145 with portions missing from pectoral, dorsal and caudal fins, was also observed on 146 other individuals. The timing of mouth damage first being observed and changes in 147 occupancy (Fig. 3), confirms that these wounds resulted from fights between males 148 over territory. On occasion, male blennies were observed emerging from their resident 149 crevice to confront a male conspecific that was moving around the reef. A typical 150 encounter would involve the two blennies facing each other a few cm apart (see Fig. 151 5a) for between 5 seconds and 1 minute before the visiting blenny swam away.

152 'Mouthing attacks" as reference by Zander (1986) were only seen very occasionally. 153 Observations by the author (PN) in both Devon and Dorset, and those reported by 154 other divers indicate that attacks involve males attempting to bite the side of the head 155 (operculum area), mouth area or the fins of their rival. In September 2014, bouts of 156 aggression were observed between juvenile P. gattorugine, approximately 25 mm 157 long (see Fig. 5b), in the same area of Devon reef where the observations on adults 158 were made. Juvenile *P. gattorugine* settle from the plankton when around 18 mm long 159 (Dunne and Byrne, 1979; Fives, 1986).

160

161 Courtship and reproduction

Male *P. gattorugine* were seen guarding eggs between April and July in their resident crevices on the Devon reef (Fig. 3). Eggs were attached to the crevice's floor, ceiling or both. Individuals were regularly observed wiping their anal/bulb glands across the eggs as the male blenny wriggled within the crevice. In many cases, eggs in different parts of the egg mass were clearly at different stages of development and had been

167 laid at different times. Females were on occasion observed within the resident 168 crevices between April and June (Fig. 3) but were never seen in the same crevice on 169 the next dive, even on subsequent days. Females within resident crevices often 170 appeared to be laying eggs on the crevice floor or ceiling, with the male in close 171 attendance (see Fig. 6a). On other occasions, females were observed outside a resident 172 crevice while the male watched from the crevice entrance (see Fig. 6b). No examples 173 of a female entering a crevice after such an encounter were observed, and the male 174 often chased off the female.

175 A remarkable example of *P. gattorugine* courtship was observed at Swanage, 176 Dorset. Two male blennies were first seen engaged in an aggressive encounter, with a 177 female blenny approaching the males. Later, the male blenny that dominated this 178 aggressive encounter moved an empty shell of a whelk (Buccinum undatum) around 179 the seabed, with a series of head movements. While the male was moving the shell, 180 two female blennies approached the male and appeared to watch this activity. One of 181 these female blennies was the same individual that had approached the males during 182 the aggressive encounter (a timeline of observations with example photographs is 183 given in Table I). In total, the whelk shell was moved on 10 separate occasions. There 184 were two main spells of shell movement by the male; the first was associated with the 185 close approach of a female blenny to the male and the second was associated with the 186 close approach of that female plus another (Fig. 7).

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188 Discussion

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190 Information on the breeding and territorial behaviour of *P. gattorugine* is currently 191 sparse and direct observations of this complex behaviour are difficult and time

192 consuming to record. In particular, there are no details on the long-term retention of 193 territory by males or courtship behaviour. The observations reported here show that 194 individual blennies can be reliably recognised over several years using photographic 195 records of distinctive skin markings around the head. Where the position and layout 196 of resident crevices permit regular close-up photography, the long-term residence of 197 individuals can then be recorded.

198 Observations on the Devon reef showed that individual male P. gattorugine 199 were regularly found occupying the same locations over more than one breeding 200 season. One male was found in the same area of reef for four consecutive breeding 201 seasons, although the exact location changed during that time. This contrasts with the 202 population of the intertidal blenny Lipophrys pholis studied by Almada et al (1992) 203 where the territories of the breeding males were temporary and established each 204 breeding season. The long-term retention of residences by male *P. gattorugine*, plus 205 some shifts and exchanges, along with the injuries sustained in territorial fighting, all 206 demonstrate that competition between males over territory is intense. Observation of 207 adult-type fighting between juvenile P. gattorugine within a few weeks of their 208 settling from the planktonic larval stage suggests that territoriality is important from a 209 very early age. It is noteworthy that, although the studied Devon reef is very exposed 210 to wave action from the south and west, occupancy of the *P. gattorugine* individuals 211 remained largely unchanged over the winter of 2013-2014 when south-west England 212 was battered by a succession of severe storms that caused notable coastal damage and 213 large movements of sediments (Hiscock, 2014).

Male *P. gattorugine* guarding eggs on the Devon reef had enlarged and very obvious anal glands when they were guarding eggs. In other blenny species, the anal glands of males produce pheromones which attract females (Barata *et al*, 2008) and

217 mucus containing anti-microbial compounds that, when applied to eggs, will improve 218 their survival (Giacomello et al, 2006; Pizzolon et al, 2010). The observations of male 219 P. gattorugine wiping expanded glands across the rafts of eggs in the current study, 220 suggest their functions include an egg-maintenance role in this species. Observations 221 reported here are consistent with previous reports of fractional spawning by female P. 222 gattorugine (Dunne and Byrne, 1979) and of males guarding eggs laid by several 223 females in a resident crevice (Zander 1986). Polygamy among related blenny species 224 is well known, with a single male attempting to ensure a number of females lay eggs 225 in his resident crevice and a female laying eggs in the resident crevices of several 226 different males (Giacomello & Rasotto, 2005).

227 Courtship interactions in *P. gattorugine* appear to be complex. On the Devon 228 reef, visits by females to resident male crevices were brief, with no female ever seen 229 in a male's residence on consecutive days. Females appeared to display to males near 230 their residences but these interactions regularly resulted in the male chasing the 231 female away. Neat and Lengkeek (2009) note that male selectivity, with aggression 232 towards females or rejection from the nest site, is commonly exhibited by other 233 blenny species. In some species, the peacock blenny (Salaria pavo) and the Azorean 234 rock-pool blenny (Parablennius parvicornis) for example, some smaller males adopt 235 alternative reproductive tactics (Oliveira et al, 2001; Ros et al, 2006). These 'sneaker' 236 males enter the nests of resident males from which they steal fertilisations. There is no 237 record of male 'sneaker' behaviour in Parablennius gattorugine but, in this little-238 studied species, it is an intriguing question whether some of the small blennies chased 239 away by resident males (such as on the Devon reef) are 'sneaker' males rather than 240 females.

241 In the Dorset observations, a bout of aggression between two male P. 242 *gattorugine* was observed by a female for part of its duration. Following the bout, the 243 dominant male manipulated a whelk shell with at least ten separate movements across 244 the seabed. This activity (outlined in Fig. 7) received the attention of one female (the 245 individual that had observed the aggressive bout) then also a second female. There 246 was no food (mollusc or hermit crab) within the shell and it is concluded that the 247 manipulation of the shell by the male represented use of a prop to display prowess to 248 the females. This appears to be the first record of prop use by teleost fish in courtship. 249 Fish have been observed using tools to access and capture food (with anvils and water 250 jets) and in nest building and care and transport of eggs. This is, as yet, limited to a 251 few observations in a small number of groups, not including blennies (reviewed in 252 Brown, 2012).

253 A further surprising aspect of the Dorset observations was that courtship 254 behaviour occurred in September, well after the expected spring to early summer 255 breeding season of this species (Dunne & Byrne, 1979; Zander, 1986; Maitland & 256 Herdson, 2009; Devon observations this study). It is not clear whether there is late 257 summer spawning in *P. gattorugine* or whether courtship behaviour occurs as part of 258 territoriality outside the egg-laying period. Territorial behaviour and male-female 259 interactions in this species have also been observed by the author (PN) at Swanage, 260 Dorset in September 2009 and at St Agnes, Cornwall in October 2013.

This study provides good evidence of territoriality and long-term retention of territory in a small number of *P. gattorugine*. Detailed data have only been collected in a single location, although the conclusions are consistent with general observations in other areas where this species is found. A number of observations support the conclusion that courtship in this charismatic species is complex. The use of a prop to

display prowess in courtship is intriguing and merits further investigation under
controlled conditions in what would no doubt prove an interesting avenue for future
work.

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