Evolutionary Ecology Research, 2019, **20**: 279–295

Sex-biased mortality of marine threespine stickleback (*Gasterosteus aculeatus* L.) during their spawning period in the White Sea

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ABSTRACT

Hypothesis: Selective sex-related mortality of the marine threespine stickleback in the White Sea causes the female-biased sex ratio observed on the spawning grounds.

Organisms: Adult threespine stickleback (*Gasterosteus aculeatus*) spawning in the inshore zone, and three species of predatory fishes: Atlantic cod (*Gadus morhua*), shorthorn sculpin (*Myoxocephalus scorpius*), and fourhorn sculpin (*Myoxocephalus quadricornis*).

Place and times: Kandalaksha Bay, White Sea, Russia; June to August 2012–2018.

Methods: The following sampling methods were used: beach seining to determine fish density and local population size, hand collection of dead fish on the spawning grounds, gill netting of predatory fishes, analysis of predatory fish stomach contents to determine the sex of well-preserved stickleback, and morphological analysis of stickleback spines to determine the sex of decomposed stickleback.

Results: The dynamics of stickleback abundance in the lagoon is explained by inshore migration of the fish to the spawning area at the beginning of the spawning period, and their subsequent departure at the end of the spawning season, with females leaving the grounds earlier than males. During spawning (5–30 June), total stickleback mortality reaches about 0.1%; the difference in the relative mortality rates of 0.0044% per day for males and 0.0030% for females is statistically significant. Mortality increases in the post-spawning period, when fish abundance in the inshore zone falls considerably, but remains at a very low level. Because of the low level of non-predation mortality, it cannot be the cause of the female-biased sex ratio observed in the White Sea stickleback population. Predation-associated mortality caused by Atlantic cod and sculpins was male-biased. Based on the stomach contents of predatory fish, the sex ratio of stickleback prey was as follows: cod, 61% males/39% females; sculpins, 82% males/18% females, which was significantly different from the population at sea (35% males/65% females). This factor alone, however, is unlikely to explain the prevalence of female stickleback in the lagoon. The eventual offshore male-biased mortality, caused by increased depletion of energy reserves during the spawning period, is probably the main reason for the observed female-biased sex ratio.

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Keywords: Atlantic cod, fourhorn sculpin, *Gasterosteus aculeatus,* mortality, population dynamics, predation, sex, sexual dimorphism, shorthorn sculpin, spawning, threespine stickleback, White Sea.

INTRODUCTION

Sex ratio is an important factor in the reproduction potential of a population (McKellar and Hendry, 2011), and its variability and evolutionary effects have been well documented (Eshel, 1975; Charnov, 1982). From the perspective of parental strategies and resource allocation, natural selection favours equal numbers of males and females (Fisher, 1930). Biased sex ratios can evolve during later developmental stages via local competition for mates and resources, affecting patterns of sexual selection (Karlin and Lessard, 1986) and the evolution of mating systems. Many aspects of mating systems, such as courting, energy investment, and parental care, are subject to discernible variation (Møller, 1994). Among fish, the threespine stickleback (*Gasterosteus aculeatus*) has been shown to be a good model for studying sex ratio, mating, and sexual selection (Milinski and Bakker, 1990; Pike *et al.*, 2007; Tinghitella *et al.*, 2013). A common fish in marine ecosystems of the northern hemisphere, the threespine stickleback is characterized by high phenotypic plasticity across its wide distribution range (Wootton, 1984). However, most data on the expression and possible advantages of stickleback sex traits were collected under laboratory conditions. It remains unclear how sexual selection is affected in wild populations in the presence of subtle ecological influences (Kraak *et al.*, 2000).

In addition, little is known about changes in the sex ratio of stickleback at different developmental stages. Since this species exhibits chromosomal sex determination, the sex ratio of progeny at birth is usually balanced (Griffiths *et al.*, 2000; Peichel *et al.*, 2004; Bakker, 2016). In most of their distribution range, stickleback reach maturation and successfully spawn at least one year after birth. In the White Sea, stickleback spend most of their first year offshore far from the spawning grounds. Mature fish appear inshore in late May to early June, approaching a density of 100 individuals per square metre (Ivanova *et al.*, 2016; Golovin *et al.*, 2017; Dorgham *et al.*, 2018). Spawning occurs mostly during the second half of June. Newly hatched larvae appear in early July and spend several weeks in the coastal zone feeding on plankton and benthos (Demchuk *et al.*, 2015). In September, the juveniles leave the inshore zone for deep offshore waters where most mature into their second year of life (Ivanova *et al.*, 2016). According to data on age composition, the proportion of males and females is equal among one-year-old fishes, but females are almost twice as common among older fish (Golovin *et al.*, 2017). The sex ratio may change with age due to sex-related mortality, in particular the marked depletion of energy reserves associated with spawning (Pike *et al.*, 2007), limited resources during overwintering (Chellappa *et al.*, 1989), selective parasitism (Arnold *et al.*, 2003), and predation (Moodie, 1972; Kraak *et al.*, 2000; Johnson and Candolin, 2017). White Sea stickleback are an important food item for common marine predators, including Atlantic cod (*Gadus morhua*), navaga (*Eleginus nawaga*), and shorthorn sculpin (*Myoxocephalus scorpius*) (Bakhvalova *et al.*, 2016). These fish are mostly demersal but prey on stickleback during the stickleback spawning season. Adult stickleback spend the rest of their lives offshore, where they are largely unaffected by these predatory fish.

Pronounced shifts in sex ratio might substantially change population dynamics. Trophic interaction studies clearly show that stickleback play a role in the circulation of nutrients and matter (Krokhin, 1970; Gislason *et al.*, 1998; Lemmetyinen, 1973; Bakhvalova *et al.*, 2016). Thus, studying their sex ratio from a long-term perspective will help to show how this particular small fish can alter the structure of marine ecosystems. Unfortunately, limited data on long-term population dynamics precludes an evaluation of the ecological role of stickleback. Also, little attention has been paid to the spatial distribution of non-spawning stickleback in large bodies of water (Gislason *et al.*, 1998; Kitano *et al.*, 2012; Ivanova *et al.*, 2016). Marine stickleback in the White Sea is a good candidate with which to address these important research questions. Based on historical data, there have been significant fluctuations in stickleback abundance at sea. A period of high abundance in the first half of the twentieth century was followed by a decline from the 1960s to the 1990s, but the population began to grow again at the end of the century (Lajus *et al.*, 2013), and is now approaching its historical maximum in the White Sea.

Using the literature and our own research, we identified sources of White Sea stickleback mortality that could cause the observed shift in population sex ratio: (1) predation and (2) non-predation mortality during the spawning period, and (3) offshore mortality throughout the rest of the year. Here, we address the first two of these, testing the hypothesis that the unbalanced sex ratio in the White Sea stickleback population is associated with increased male mortality during the spawning period, caused by the depletion of energy reserves associated with spawning, and male-biased predation by demersal fish.

METHODS AND MATERIALS

Sampling sites

The study was conducted at three sites in Kandalaksha Bay in the White Sea, representing inshore spawning grounds of different quality – Koliushkovaya Lagoon, Seldianaya Inlet, and Sukhaia Salma Strait (Fig. 1). Non-predation mortality was studied in Koliushkovaya Lagoon, as this is a shallow and relatively isolated water body with spring tides raising the water level to only 0.3 m above average; during neap tides the lagoon's surface is above sea level. The bottom of the lagoon is relatively flat and muddy with seagrass and filamentous algae in the shallows. In summer, water temperature reaches 22°C, higher than that in the other two locations. The salinity depends on the amount of rainwater mixing with incoming seawater and is usually slightly lower than in the open sea (15–20 ppm).

Predation-associated mortality was studied in both Seldianaya Inlet and Sukhaia Salma Strait. Seldianaya Inlet is characterized by a wide opening connecting it with the sea and is approximately 3–4 m deep. Water exchange during tides is strong, and the salinity – at around 23–24 ppm – is the highest among the sampling sites. Bottom sediments are characterized by mud and sand in the sublittoral zone and rocks in the littoral zone. This location represents typical marine habitat with high-density seagrass beds (Dorgham *et al.*, 2018; Lajus *et al.*, 2019). In summer, the water warms to between 12 and 20°C (Dorgham *et al.*, 2018), and the feeding conditions are so favourable that stickleback density exceeds 3000 individuals per square metre (Rybkina *et al.*, 2017).

The last site, Sukhaia Salma Strait, is characterized by strong tidal currents and weak solar heating. Average salinity is a little higher than in the lagoon, and inflow from nearby rivers (e.g. River Keret) is enough to cause significant desalination. Sparse eelgrass beds are unevenly distributed on the muddy sublittoral bottom, and a few fucoids are found on stony areas of the littoral zone. Both Seldianaya Inlet and Sukhaia Salma Strait host stickleback predators (Dorgham *et al.*, 2018; Lajus *et al.*, 2019).

Fig. 1. Sampling sites in Kandalaksha Bay in the White Sea.

Stickleback abundance and non-predation mortality in the Koliushkovaya Lagoon

To assess the number of stickleback in the lagoon, we used a 7-m beach seine in 2012 and 2015–2018.

In order to assess fish density, samples were taken at 10 stations, evenly distributed around the lagoon, at a distance of approximately 100 m from one another (for a map, see Ivanova *et al.*, 2019). Since all stations could not be sampled evenly each year, the data were averaged over several years for each station. Average densities were later used to calculate the total number of fish in the lagoon. Where data were available for several years, the inter-annual variability at those sites was also investigated. This was necessary to justify the averaging of data over several years and using sites with a small number of observations (see description of ANOVA below).

We used data acquired during the spawning season (5–30 June) from 2015 to 2018, obtained from 46 beach seine hauls at 10 stations. For the post-spawning period (after 30 June), we used data from 2012 and 2016 obtained from 17 beach seine hauls at four stations. Inter-annual variability in abundance during June spawning was estimated based on data from four years, four stations, and 37 hauls; during the post-spawning period in July–August, data were based on two years, one station, and seven hauls. Data relating to sampling structure are presented in detail in the Appendix (evolutionary-ecology.com/data/3163Appendix.pdf).

The total area of the lagoon is 58,000 m^2, and the area available for beach seining (i.e. within 30 m from the shoreline) is 23,200 m^2. This is where 92% of all fish in the lagoon are found because most stickleback prefer shallower coastal areas (our unpublished data). The absolute number of stickleback in the lagoon was estimated using formula (1):

$$N_{abs} = \frac{N \times S}{0.92}, \tag{1}$$

where N_{abs} is the absolute number of stickleback in the lagoon (individuals), N is the average density of stickleback (individuals/m^{-2}) from 10 sites during the spawning period and four sites during the post-spawning period, S is the catch area of the beach seine (m^2), and 0.92 is the ratio of the number of fish in the catch area to the total number of fish in the lagoon. The sex ratio was determined from samples comprising 150–200 individuals.

Due to the lagoon's isolation and weak tides, all dead fish remained within the lagoon. Dead stickleback were collected along the entire shoreline of the lagoon from June to August 2016–2018 at 2–12 day intervals within about 5 m of the shore. Observations from our boat suggested few dead fish outside this 5 m zone. Scavengers that consume dead fish, such as large snails, starfish, and crabs, are absent from the lagoon. Seabirds such as seagulls appear there infrequently, likely because spawning stickleback are more plentiful elsewhere nearby.

In most cases, sex was determined visually. Typically, spawning males have bright red throats and blue eyes, while females are greyish all over. If there were difficulties in determining sex, the fish were dissected. Standard length was measured from a photo of the fish using the free software, ImageJ.

Two indicators were used to characterize fish mortality:

1. Daily fish mortality (number of dead individuals per day) was estimated as the number of dead fish divided by the number of days since the previous census.
2. Relative mortality was calculated as daily fish mortality divided by the total number of fish in the lagoon during the same period.

Predation-associated mortality

We caught predatory fish [Atlantic cod, shorthorn sculpin, fourhorn sculpin (*Myoxocephalus quadricornis*)] in Kandalaksha Bay during the summers of 2015–2016 with gill nets in the coastal zone near the stickleback spawning grounds. The stomachs of the predators were dissected, and the undigested remains of individual stickleback were identified and counted. During analysis, we pooled data for the two species of sculpin.

In about 75% of cases, the devoured stickleback were in poor condition. Only the bones remained, thus we could not determine sex by body coloration or gonads. Using a description of sexual differences in the shapes of stickleback dorsal spines (Kitano *et al.*, 2007), we developed a novel technique for sex determination, which is described in the following section. Initial preparation included complete extraction of the bony structures, which were cleaned with a needle in a Petri dish filled with water. To measure predator preference for male and female stickleback, we calculated the index of electivity, E (Ivlev, 1955; Wootton, 1984):

$$E = \frac{r_i - p_i}{r_i + p_i}, \tag{2}$$

where r_i is the relative content of food item i in the stomach of a predator, and p_i is the relative content of food item i in the environment.

A method for determining stickleback sex based on dorsal spines

Since in most cases only bones remained in the stomachs of the predatory fish we sampled, we developed the following method for determining the sex of individual stickleback using spines. First, we created a scale of dorsal spine serrations based on five gradations (Fig. 2). Then, we tested the scale against a sample of randomly selected individuals from Seldianaya Inlet: 28 males and 30 females, the sex of which was determined by gonads. Spine structures were studied under a microscope (MBS-10), and each spine was assigned a serration score from 1 (minimal serration) to 5 (marked serration) (Fig. 2). We conducted a blind analysis in relation to sex and to spine order (first or second), i.e. the operator scoring the serration of a particular spine did not know the sex of fish or whether it was the first or second spine. After two days, the scoring was replicated to assess measurement error. We compared the scores assigned to the first and second spines, and the scores from the first and second observations of each spine.

Statistical analyses

All statistical analyses were done using the StatSoft Statistica v.10.0 and Microsoft Excel 2013 software packages. To assess fish abundance during the spawning period, we applied main effect analysis of variance (ANOVA) to densities of fish at four sites, with 'year' and 'site' as factors. Each site was sampled at least three times over 2–4 years. In the post-spawning period, divided into two intervals (1–5 July and 6–25 July), the factors 'year' and 'interval' were used; we used data from two years (2012 and 2016) and one site. These preliminary analyses were used only for assessment of stickleback abundance in the lagoon. Comparisons of daily fish mortality and relative mortality were performed using two-way ANOVA (factor combinations: 'year' and 'sex'; 'spawning/post-spawning period' and 'sex'). Comparing the sex ratio of dead and live fish was performed using Pearson's χ^2 test.

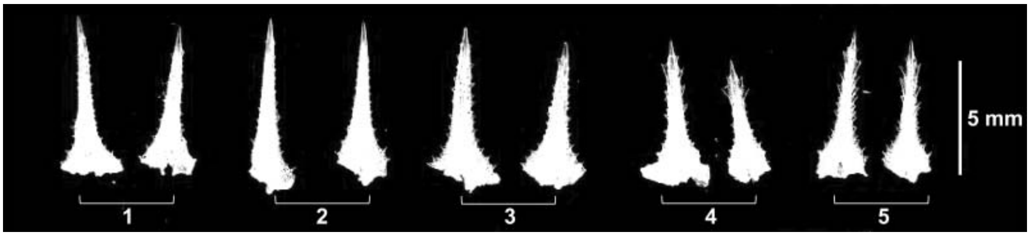

Fig. 2. Threespine stickleback spine serration scale (the first and second spines are shown in pairs). Spines were rated for extent of coverage by notches, the frequency of notches, and notch size. Scale: 1 = few small notches, barely visible, and absent at the end of the point; 2 = few small notches and absent at the end of the point; 3 = few average-size notches distributed along the whole spine, including the point and the triangular base; 4 = frequent large notches unevenly distributed along the whole spine, forming a 'crown' around the point; 5 = large notches evenly distributed along the whole spine, including the point.

RESULTS

Non-predation mortality

Inter-annual variation of stickleback density during the spawning period at different sites in the lagoon was not statistically significant (ANOVA, 'year': $F_{3,30} = 0.17$, $P = 0.92$). Although we observed significant spatial heterogeneity (ANOVA, 'site': $F_{3,30} = 6.00$, $P = 0.0025$) (Fig. 3), this was not the focus of this study. There was no significant inter-annual variation in density between samples collected post-spawning (July and August) during 2012 and 2016 (ANOVA, 'year': $F_{1,3} = 0.23$, $P = 0.67$). However, significant differences were observed between the two parts of the post-spawning period: 1–5 July and 6–25 July (ANOVA, 'interval': $F_{1,3} = 17.7$, $P = 0.024$).

The dynamics of stickleback abundance in the lagoon, drawn from observations over several years, are as follows. During spawning, stickleback abundance and sex ratio change little: abundance = 200,000–250,000 fish, 70% of which are females (Table 1). Female abundance begins to fall just after spawning, decreasing by two-fold during the first week and more that 30-fold by the second half of July. Males that care for the progeny leave later and at a slower rate; their numbers fall three-fold by the second half of July. In August, only a few hundred fish of both sexes remain in the lagoon.

Table 2 shows the number of dead fish collected during our surveys. No differences were observed across years in the daily mortality of both the spawning and post-spawning periods (ANOVA, spawning: $F_{1,16} = 2.33$, $P = 0.15$; post-spawning: $F_{2,10} = 1.08$, $P = 0.38$), or in the relative mortality of the two periods (ANOVA, spawning: $F_{1,16} = 3.96$, $P = 0.064$;

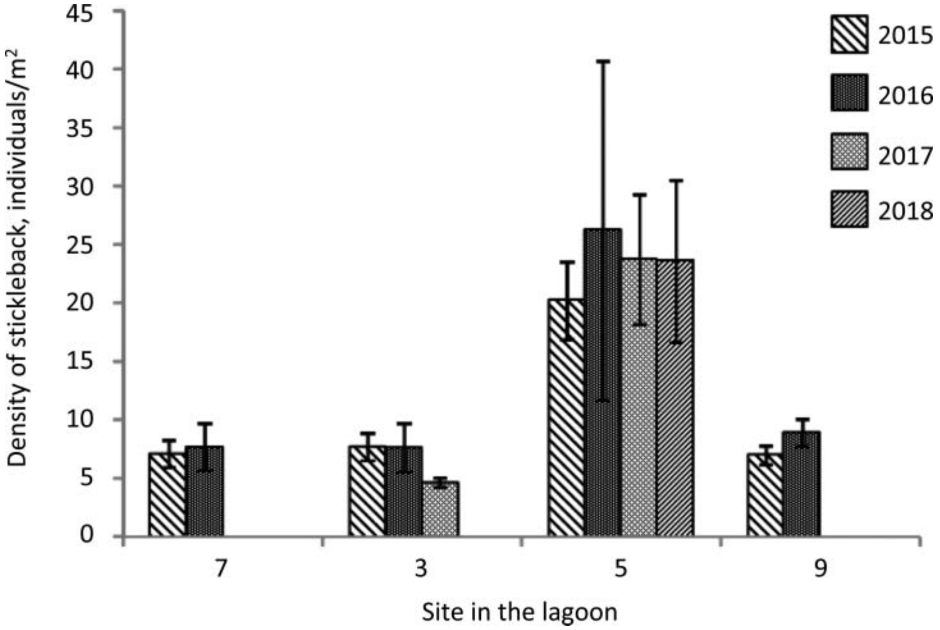

Fig. 3. Stickleback density based on beach seine sampling (37 hauls) at different sites in the lagoon, 2015–2018 [for a map, see Ivanova *et al.* (2019)].

Table 1. Estimates of stickleback density and mortality in Koliushkovaya Lagoon based on data from 2012 and 2015–2018 (mean ± S.D.)

	Spawning period	Post-spawning		
	5–30 June	1–5 July*	6–25 July	26 July–10 August**
Density of stickleback, *n*/m^2 (% male)	9.2 ± 1.9 (30%)	6.3 ± 2.2 (51%)	1.1 ± 0.6 (84%)	
Absolute no. of stickleback ($n \times 10^3$)				
males	69.6 ± 14.5	81.3 ± 27.7	23.3 ± 11.8	0.2–0.4
females	161.9 ± 33.8	78.1 ± 26.6	4.4 ± 2.2	
Absolute mortality (*n*/day)				
males	3.1 ± 0.56	—	4.5 ± 2.3	3.3 ± 2.13
females	4.8 ± 0.98	—	5.6 ± 2.09	2.8 ± 2.13
Number of dead fish (*n*)				
males	77 ± 14	—	89 ± 46	52 ± 34
females	120 ± 24	—	113 ± 42	44 ± 34
Relative mortality (%/day)				
males	0.0044 ± 0.0008	—	0.0191 ± 0.0099	—
females	0.0030 ± 0.0006	—	0.1283 ± 0.0475	—
Dead fish as percent of population				
males	0.11 ± 0.02	—	0.38 ± 0.20	—
females	0.07 ± 0.02	—	2.57 ± 0.95	—

* Mortality was not assessed. **Mortality estimates are not used for analyses of relative mortality due to low reliability of the estimate of stickleback abundance.

Table 2. Numbers of dead fish collected in the Koliushkovaya Lagoon, 2016–2018

Period	Year	Date	Days since previous census	Number of dead fish, individuals		Daily mortality, individuals/day	
				Males	Females	Males	Females
Spawning	2016	10 June 2016	5	25	18	5.0	3.6
		12 June 2016	2	3	2	1.5	1.0
		15 June 2016	3	5	12	1.7	4.0
		20 June 2016	5	12	33	2.4	6.6
		26 June 2016	6	5	15	0.8	2.5
		29 June 2016	3	7	23	2.3	7.7
	2017	30 June 2017		1	3		
	2018	11 June 2018	6	12	4	2.0	0.7
		16 June 2018	5	21	21	4.2	4.2
		20 June 2018	4	22	35	5.5	8.8
		25 June 2018	5	27	46	5.4	9.2
Post-spawning	2016	11 July 2016	12	19	42	1.6	3.5
	2016	14 July 2016	3	7	14	2.3	4.7
	2017	10 July 2017	10	13	35	1.3	3.5
	2017 2018	17 July 2017	7	108	109	15.4	15.6
		25 July 2017	8	44	46	5.5	5.8
		24 July 2018	19	11	17	0.6	0.9
	2017	2 Aug 2017	8	43	39	5.4	4.9
	2017	10 Aug 2017	8	9	5	1.1	0.6

post-spawning: $F_{2,6} = 1.19$, $P = 0.37$). This allowed us to pool data obtained in different years.

Absolute daily mortality of 3–5 individuals a day was stable during our entire observation period. Throughout spawning, the percentage of dead fish was only 0.1% of all fish in the lagoon. Differences between males and females were not statistically significant for either absolute or relative mortality (*t*-test, $P = 0.14$ and $P = 0.16$, respectively). However, the sex ratio of dead fish during spawning differed from that of live fish: 60% of dead fish were female, whereas 70% of live fish were female, i.e. the relative mortality of males was significantly higher and exceeded female mortality by about 1.6-fold (Pearson's χ^2 test, $P = 0.0001$).

Absolute mortality of 4–6 fish a day in July did not differ significantly from that in June (ANOVA: $F_{1,32} = 0.22$, $P = 0.64$), and no difference was observed between males and females (ANOVA: $F_{1,32} = 1.02$, $P = 0.32$). For July, mortality data are from 2016–2018, but abundance data are based on 2012 and 2016. Thus, our relative mortality estimates are less reliable than those for the spawning period, but as calculations estimate a rate of 0.15% per day, we conclude that relative mortality post-spawning was also very low. Given that the absolute mortality rate was similar during and after spawning, but fish numbers declined drastically, one can infer that mortality increased after spawning. In addition, the sex ratio of live to dead fish was different after spawning: females comprised 20% of live fish but 67% of dead fish (Pearson's χ^2 test, $P = 0.0001$), i.e. female mortality exceeded that of males eight-fold. Mortality increased further in August, when we found about 100 dead fish in the lagoon among only a few hundred live fish. The body length (standard length ± S.E.) of males ($n = 135$) was 54.2 ± 0.4 mm (range 44–66 mm), and that of females ($n = 235$) was 60.0 ± 0.3 mm (range 45–72 mm).

Predation mortality

The first and second replicates gave the same score in 87% of measurements (81% for the first spine and 93% for the second spine, i.e. assessing the extent of serration of the second spine was more reliable than assessing that of the first spine). Scores from the first and second spines coincided in 75% of cases. The second spine was significantly more serrated than the first spine (*t*-test, $P = 0.009$). From our analyses, most females had a score of 1, 2 or 3, whereas most males had a score of 4 or 5 – thus males displayed more pronounced serration. Sex determination based on spines was correct for 27 of 28 males and for 26 of 30 females. Three of the females for which sex was determined incorrectly were the largest individuals in the sample.

Of the 238 predatory fish we analysed from Seldianaya Inlet and Sukhaia Salma Strait, 38 had stickleback in their stomachs (Table 3). The proportion of male stickleback in the stomachs of our sample predatory fish was considerably higher than we found on the spawning grounds in Seldianaya Inlet and Sukhaia Salma Strait (Fig. 4). While about one-third of the stickleback on the spawning grounds were male, they accounted for 60% of stickleback in cod stomachs and more than 80% in sculpin stomachs. The electivity index

Table 3. Information on numbers, body size, and presence of stickleback remains in the stomach contents of predatory fish sampled from Seldianaya Inlet and Sukhaia Salma Strait

Species	No. of predatory fish collected	No. of predatory fish with stickleback remains	Length (SL) of predatory fish with stickleback remains	Mean no. of stickleback per stomach
Cod	169	24	93–460 mm	1.583
Sculpins	69	14	98–270 mm	2.357

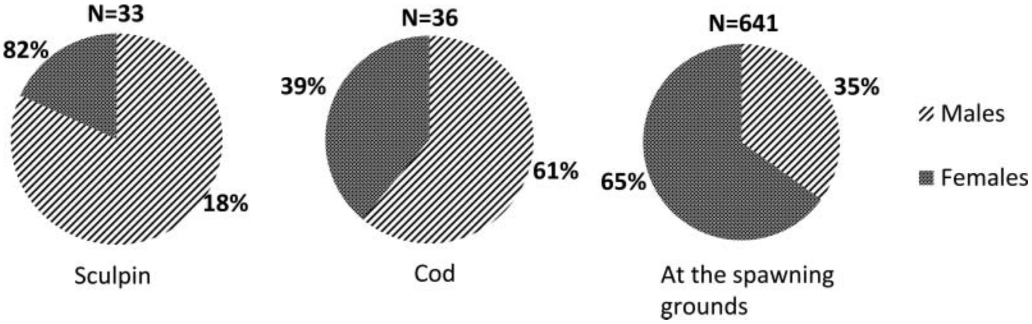

Fig. 4. Proportions of female and male stickleback in predatory fish stomach contents and on the spawning grounds. N = the total number of stickleback in a sample.

for males was 0.401 in sculpin and 0.272 in cod (Pearson's χ^2 test, sculpins: $P = 0.016 * 10^{-6}$; cod: $P = 0.001$), i.e. the predatory preference for males was statistically significant.

DISCUSSION

Non-predation mortality

In the lagoon where we performed this study, the dynamics of stickleback abundance can be explained by the gradual inshore migration of fish to the spawning area at the beginning of the spawning period, and the subsequent departure of spent fish at the end of the season. After spawning, females leave earlier because their reproductive role is limited to the release of eggs, whereas males care for the offspring. The predominance of females on spawning grounds in the White Sea may be explained either by their prevalence in the population, or by the rotation of males on spawning grounds. In the latter case, male rotation might allow for more effective use of spawning grounds, especially given the dearth of high-quality spawning grounds – in particular, seagrass beds (Rybkina *et al.*, 2017) – likely limits stickleback abundance in the White Sea (Ivanova *et al.*, 2016). Notwithstanding the possible rotation of males observed in some parts of the species range (Wootton, 1984), and their ability to nest multiple times per season (Mori, 1993), male rotation is unlikely to occur in the White Sea, where the spawning season is limited to about one month. This approximates the duration of one male stickleback's reproductive cycle even at lower latitudes (Kynard, 1978; Whoriskey and FitzGerald, 1994). In several freshwater populations that inhabit relatively shallow ponds, such as Inverleith Pond in the UK (Arnold *et al.*, 2003) and Lake Boulton in Canada (Reimchen, 1980), differences in the spatial distribution of males and females has been observed. According to our gill net catches, a small number of fish during the spawning period, predominantly females, resided some hundred metres from the shore. This suggests that females, rather than males, may rotate in and off the spawning grounds (our unpublished data). Thus, spawning migrations of males and females likely occur at the same time, similar to mid-twentieth-century reports for the White Sea (Mukhomediyarov, 1966). These considerations may indicate that the sex ratio of White Sea stickleback on spawning grounds reflects the population's sex ratio.

Stickleback exhibit different sex ratios across their distribution range, which can be stable or variable during the year (Moodie, 1972; Reimchen, 1980; Mori, 1993). Simulation modelling shows that sex ratio, if not interrupted by environmental factors, should be male-biased, favouring competition and mate choice (Wootton *et al.*, 1995). However, this is not the case in the White Sea. The high prevalence of females we observed might increase population fecundity where high-quality spawning grounds are strictly limited, as in the White Sea (Ivanova *et al.*, 2016), and where males still compete for the best spawning grounds (Dorgham *et al.*, 2018).

The increase in stickleback mortality after spawning might be evidence of 'fatigue' (i.e. the high cost of breeding), which has been reported previously (Wootton, 1984; FitzGerald *et al.*, 1989). During the inshore stage of their life cycle, the mortality of males exceeds that of females, which may indicate that male energy expenditure represents a greater risk to life. That males expend more energy than females is supported by results from biochemical examinations of relationships among the various components of energy stores (Huntingford *et al.*, 2001). We also observed more pronounced weight loss in White Sea stickleback males than in females (Golovin *et al.*, 2017; Demchuk *et al.*, 2018). A negative relationship between pre-spawning investment in gonads and in other somatic male tissues suggests that male energetic reserves begin to be used up even before the fish are in full breeding condition (Chellappa *et al.*, 1989). That male mortality shifted to earlier periods of the breeding season compared with that of females seems to confirm this finding. At the same time, studies in the White Sea of the responses of spawning stickleback males to starvation demonstrated that they can quickly restore depleted lipids (Bakhvalova *et al.*, 2018), likely indicating a special adaptation to mitigate the consequences of severe stress associated with male competition for territory and protection of progeny. The high proportion of dead females at the end of breeding season may be because robust females have already departed, leaving the weakest females on the spawning grounds at the end of the season. Most of the fish that remain in the lagoon probably die, although occasionally stickleback caught in the lagoon over winter survive under the ice (our unpublished data).

We did not find any significant difference between the size of dead and live fish. Average length of fish in our study (54.2 mm for males and 60.0 mm for females) did not differ notably from the average length of live fish from the lagoon in 2016 [57.1 mm for males and 62.8 for females (Dorgham *et al.*, 2018)] and in 2006 [55.0 for males and 59.0 for females (Ivanova *et al.*, 2007)].

In general, non-predation mortality on spawning grounds is negligible (fraction of a percent) and is highly unlikely to influence population abundance and its sex ratio. Kynard's (1978) work at Wapato Lake, USA revealed that stickleback post-spawning mortality had no discernible effect on their sex ratio. In the first post-spawning mortality peak, Kynard observed a balanced sex ratio similar to that in the population (44.5% males and 55.5% females). At the second peak, a significant increase in female mortality was observed (7.5% males and 93.5% females), but the total post-spawning mortality did not exceed 5% of the total fish. However, much higher mortality may occur. For example, in the anadromous population of the Kamchatka River (Russia, Pacific coast), almost all mature fish die after spawning (Bugayev *et al.*, 2007). In the freshwater population of the River Kelvin in Scotland, most males die, likely due to the high energy expenditure associated with spawning during wintering (Chellappa *et al.*, 1989). Dead fish are not usually observed in high numbers after spawning on other grounds in the White Sea, likely due to strong tidal events or starfish predation, which does not allow estimates of mortality to be made there. However, there is no reason to assume that mortality in these habitats differs markedly from that in the studied lagoon.

Predation mortality

A method of stickleback sex determination based on dorsal spines

That the dorsal spines of stickleback males are more serrated than those of females has previously been noted in the literature (McLennan and McPhail, 1990; Kitano *et al.*, 2007). Our analyses of samples allowed us to quantitatively determine the amount of serration, and use those results to determine the sex of fish in predator stomach contents in the absence of other traits.

Although discrepancies between the first and second spine estimates were on average 25%, these did not lead to a different sex assignment for a single fish. Good agreement between the results of repeated analyses suggests that our serration scores accurately describe the features and shape of the structure. When we verified sex of fish by coloration and gonads, the accuracy for both sexes was more than 90%. The similar proportions of incorrectly determined females and males suggests that there was no systematic error. By quantifying the differences between male and female dorsal spines, we confirmed previously published results, and developed a reliable method for identifying the sex of individual stickleback.

Patterns of mortality caused by predatory fish

Analysis of predatory fish stomach contents showed clear male-biased stickleback mortality. Several factors increase the risk of predation for stickleback males: (1) high energy expenditure associated with guarding progeny (Van den Assem, 1967); (2) bright coloration that attracts predators (Moodie, 1972; Whoriskey and FitzGerald, 1985); and (3) attachment to their nesting territory. At the same time, these factors may have selective advantages. The more energy males spend protecting their progeny, and the more territorial they are, the higher the probability of their progeny surviving. Bright red coloration might be an important factor in female mate selection (Milinski and Bakker, 1990; Smith *et al.*, 2004; Pike *et al.*, 2007), and male life span is positively correlated with intensity of coloration (Pike *et al.*, 2007). Therefore, factors increasing male predation risk also provide selective advantages.

Despite our experimental results suggesting that predation may cause a female-biased sex ratio, it is difficult to evaluate the contribution of predation to the overall sex ratio of stickleback in the White Sea without direct estimations of inshore predatory fish density. Yet, observations can still be made. First, we observed a clear shift towards females in two-year-old fish, the majority of which were not present on the spawning grounds during their first year (Ivanova *et al.*, 2016). Second, during the spawning period, we did not observe any shift in sex ratio towards females; indeed, the proportion of males grew continuously during the spawning period. Although these arguments do not constitute proof, they suggest that predation during spawning periods alone is unlikely to explain the existing shift in sex ratio towards females. Given that non-predatory mortality during the spawning period is negligible, the only alternative is mortality during the offshore phase of the stickleback life cycle.

Therefore, we conclude that non-predation and predation mortality on spawning grounds – both male-biased – are, nevertheless, unlikely to explain the observed prevalence of females in the White Sea stickleback population. Predator-associated mortality is negligible due to the enormous number of stickleback compared with the numbers of its major predators, although such mortality may rise during periods of low stickleback abundance in the White Sea. Non-predation mortality during the spawning period is also negligible. This may indicate that the observed female-biased mortality patterns persist after the fish leave the spawning grounds for offshore areas. In this case, offshore male-biased mortality, probably caused by the males' marked depletion of energy reserves during the spawning period, presents a reasonable explanation of the observed sex ratio. Offshore mortality was observed by V. Kuznetsov, who found dead stickleback in trawl samples in Kandalaksha Bay at depths of up to 80 m (Andriyashev, 1954), but quantifying it will require more research, particularly during the offshore period of the life cycle of White Sea stickleback.

CONCLUSION

This study explored the threespine stickleback as a key species within the White Sea ecosystem. As the most abundant species in the White Sea (Ivanova *et al.*, 2016), stickleback population ecology (Lajus *et al.*, 2013; Golovin *et al.*, 2017; Dorgham *et al.*, 2018; Murzina *et al.*, 2018, 2019; Ivanova *et al.*, 2019; Lajus *et al.*, 2019) and its biotic interactions with other species (Demchuk *et al.*, 2015, 2018; Bakhavalova *et al.*, 2016; Rybkina *et al.*, 2016, 2017) are important for understanding mechanisms of change in the ecosystem as a whole.

Understanding the causes of stickleback mortality is important both from an autecological and synecological standpoint. The factors responsible for male-biased mortality, such as marked depletion of energy reserves during the spawning period, and their increased vulnerability to predators may eventually shift the sex ratio towards females. Yet, a female-biased population, in which males protect the progeny, more effectively utilizes opportunities in the White Sea environment, where high-quality spawning grounds are limited but open-sea feeding resources are likely not (Ivanova *et al.*, 2016). Therefore, patterns of White Sea stickleback population mortality apparently conform with the structure of sea biotopes, and may be responsible for the quick growth of stickleback in the 1990s and 2000s (Lajus *et al.*, 2013) and their current high abundance in the White Sea.

ACKNOWLEDGEMENTS

The authors thank Karen Alexander for improving our English and the administration of the White Sea Biological Station of the St. Petersburg State University for the opportunity to conduct year-round research in the White Sea. This work was supported by grant #18-34-00914 from the Russian Foundation of Basic Research.

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