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Predation of threespine stickleback (*Gasterosteus aculeatus*) on the eggs of Atlantic herring (*Clupea harengus*) in a Baltic Sea lagoon

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Abstract

We analyzed the predation of threespine stickleback (*Gasterosteus aculeatus*) on the spawn of Atlantic herring (*Clupea harengus*) in a mesohaline lagoon of the Baltic Sea, hypothesizing a significant predation effect of a resident estuarine fish on the recruitment of an anadromous oceanic species. A predator exclusion field experiment using artificially spawned experimental units was combined with tank feeding experiments to estimate the effects of temperature and prey density on the herring egg consumption by estuarine *G. aculeatus*. The predator exclusion experiment showed a significant mortality of herring eggs caused by estuarine predatory fish species. A strong increase in the consumption of herring eggs by sticklebacks was observed between 11°C and 15°C (Q_{10} = 3.15). Additionally, we found a significant positive correlation between egg concentration per area and predation intensity. Nearly all eggs were consumed at concentrations ≥ 25 eggs cm^{-2}, but predation was less intense at egg concentrations below that threshold. Field data on herring egg concentrations, stickleback abundances, and stomach contents were combined with the findings of the experiments to estimate the percentage of spawned herring eggs that is consumed by the local stickleback (M_{PS}). The highest M_{PS} (11.4%) was estimated for a week in April in the second half of the spawning season. We conclude that stickleback predation on herring eggs potentially affects the local herring year class strength.

Transitional waters connecting temperate river tributaries with the coastal ocean worldwide represent nutrient-rich, mesohaline, and seasonally highly variable environments. These characteristics pose unique challenges for euryhaline faunal communities populating those ecosystems and also for scientists investigating the ecology of these waters (Elliott and Whitfield 2011). Fish communities of temperate estuaries and coastal lagoons are often subject to significant changes during the course of a year (Thiel et al. 1995). This variability is caused by seasonal changes in environmental conditions, the specific migration behaviors, and the reproductive cycles of the different species. Ecological studies of qualitative and quantitative aspects of estuarine fish assemblages therefore often represent merely a temporary status of an otherwise highly dynamic system. These snapshots are difficult to generalize, e.g., in order to construct predictive multispecies model approaches for ecosystem productivity or energy transfer. Few studies include changes in estuarine fish assemblages caused by seasonal immigration of oceanic species, and even fewer consider the actual extent and importance of trophic links generated in the context of this migratory behavior.

Western Baltic spring-spawning herring (*Clupea harengus*) undergoes an extensive annual migration (Aro 1989). Spending most of the year within offshore regions, this particular stock migrates to mesohaline shallow estuaries for spawning. Baltic spring-spawning herring prefers shallow coastal spawning beds, where the adhesive eggs are attached to complex benthic substrates such as submerged aquatic vegetation (Scabell and Jönsson 1984; Haegele and Schweigert 1985). Like herring in the Baltic Sea (Aneer 1989), Pacific herring (*Clupea pallasi*) spawns demersally in the shallow areas of lagoons and sounds in the northern Pacific (Haegele and Schweigert 1985; Hoshikawa et al. 2004). Although it is commonly known and often documented by public media and television that even terrestrial predators such as bears (*Ursus* sp.) visit the Pacific shoreline of North America to feed on herring eggs in the intertidal zone, scientific studies on these trophic interactions are rare or difficult to access. Most investigations on the predation mortality of Pacific herring spawn survival focus specifically on avian predators (Bishop and Green 2001; Anderson et al. 2009). Little is known about predation effects of local fish communities (but *see* Rooper and Haldorson 2000). In the intertidal area where herring eggs are exposed not only to diving birds but to the entire waterfowl community, egg predation might indeed be mainly driven by birds. In contrast, many spawning beds of Atlantic herring remain permanently submerged, owing either to the lack of significant tidal amplitudes (Baltic Sea stocks) or to spawning grounds located farther offshore in deeper waters (North Atlantic stocks). Thus, egg predation by non-diving birds, e.g., seagulls, on these stocks can rather be neglected. Although there are several studies demonstrating that diving ducks such as long-tailed duck (*Clangula hyemalis*) feed on herring spawn in coastal waters (Jamieson et al. 2001; Zydelis and Esler 2005), the importance of piscine predators might be elaborated in those spawning grounds. Demersal fish species, particularly haddock (*Melanogrammus aeglefinus*), are considered to have a significant effect on the survival of herring eggs on outer-coastal-shelf spawning beds of the North Atlantic Ocean (Rankine and Morrison 1989; Toresen 1991).

Although herring has been the subject of biological science and resource management for more than a century, trophic interactions of Baltic herring with the resident

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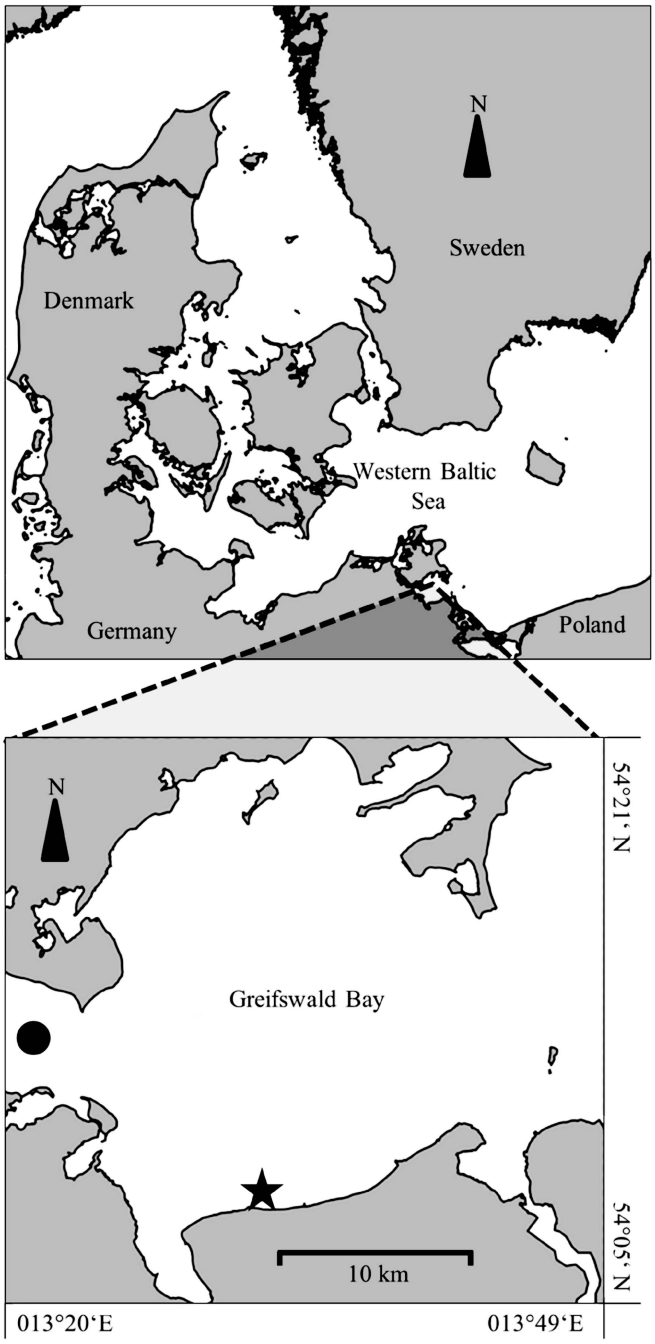

Fig. 1. Greifswald Bay and its location within the Western Baltic Sea. The black star indicates the position of the fixed transect sampled in 2011, the location of the exclosure experiment in 2012 and the area where wild threespine stickleback were caught for the gastric evacuation experiments in 2012. The black circle represents the position of the gillnet fishery on ripe and running herring as the source of eggs for attachment to experimental units.

faunal community in the inshore spawning areas remain widely unknown. Most studies on herring egg predation in the Baltic Sea also focus on bird predation (Stempniewicz 1995), whereas literature on the effects of piscine predators on Baltic herring egg mortality is limited (Scabell 1988; Rajasilta et al. 1993). Furthermore, these few existing studies report side observations rather than empirically evaluating the effect on herring egg survival that may arise from trophic links to the resident fish community. On the spawning beds of Baltic herring, egg concentration and abundance of potential predators such as threespine sticklebacks (*Gasterosteus aculeatus*) can simultaneously reach high levels (Scabell 1988; P. Kotterba unpubl.). The trophic link that may arise from this overlap might have multiple effects. The predators might influence the annual reproduction of Baltic herring, which is crucial for stock management purposes, while the resident predator community might benefit from the massive supply of energy-rich and easily accessible food.

G. aculeatus is the dominant resident fish species in Baltic Sea inshore waters (Winkler and Thiel 1993; Nilsson et al. 2004). It is known to feed on fish spawn in general (Nilsson 2006), and side notes from earlier studies also indicate a predation on herring eggs by the threespine stickleback (Soin 1971; Scabell 1988). The high stickleback abundance on herring spawning beds and its opportunistic feeding behavior pronounce the key role of estuarine stickleback for analyses of trophic links between Baltic herring and the inshore fish community.

In this study, we analyzed basic trophodynamic parameters such as consumption rates and prey density dependence and combined them with field observations on abundances and feeding behavior of potential predators. We hypothesize that stickleback predation is an important source of mortality for herring eggs and a potential mechanism controlling the year class strength. Furthermore, we hypothesize that the predation behavior is not independent of herring egg concentration.

Methods

Study area—We focused our investigations on Greifswald Bay, a typical Baltic inshore lagoon (Fig. 1), which is an important spawning area of spring-spawning herring of the western Baltic Sea (Scabell 1988). The semi-enclosed bay comprises an area of $\sim$ 514 km^2 and is characterized by a mean depth of 5.8 m with a maximum of 13.6 m (Reinicke 1989). Because tidal amplitudes are marginal ($<$ 10 cm) in the inner Baltic Sea region, water exchange and sea level amplitudes at the study site are mainly wind driven (Stigge 1989). The system is mesohaline, with mean salinities of $\sim$ 6 in spring and summer and 8 in winter. Although the ecosystem is highly eutrophic (Munkes 2005), frequent wind-driven mixing of the shallow water results in high oxygen levels even close to the bottom. The aquatic vegetation within the shallow littoral zone is permanently submerged and consists of flowering plants such as pondweeds (Potamogetonaceae) and eelgrass (*Zostera marina*), as well as a diverse macroalgal community (Geisel and Meßner 1989). Winkler (1989) described 61 freshwater as well as marine and mesohaline species forming the fish community of Greifswald Bay.

Field observations—In spring 2011, a major spawning bed in Greifswald Bay was monitored every second week for

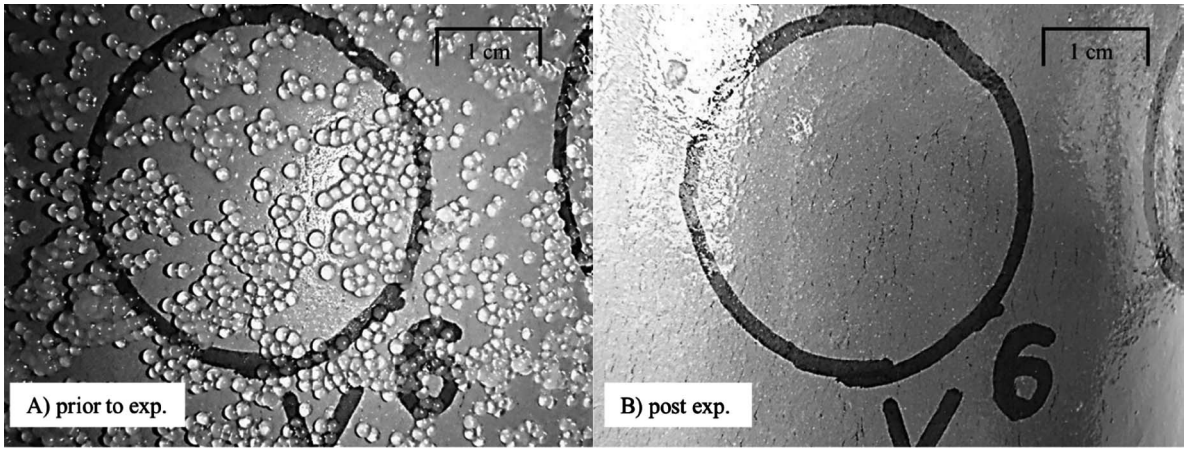

Fig. 2. Predefined subarea situated on an ASEU of the uncaged control group of the predator exclosure experiment. (A) shows a digital image taken before the exposure (exp.) to predators to quantify egg concentration. (B) shows the particular subarea after 72 h of exposure in the field, resulting in a 100% removal of herring eggs.

herring egg concentrations and abundances of threespine sticklebacks (Fig. 1). Spawn concentrations were estimated using random counting frame samples ($n = 6$) alongside a fixed transect of 100 m at a mean water depth of ~ 1.2 m. A randomly chosen subsection of 12.5 × 12.5 cm of the total counting frame area (0.25 m^2) was completely harvested, including herring spawn and all available spawning substrate. In the laboratory, the spawn was separated from the substrate and the total dry weight of herring eggs was measured after incubation for 48 h at 80°C. A beach-seine (10 m total length, 7 m mouth opening, and 5 mm mesh size) was applied to estimate the abundance of sticklebacks. The net was towed manually over a distance of ~ 100 m. The exact area swept was calculated by multiplying the distance between start and end positions derived by a Global Positioning System receiver (Garmin etrex VISTA HCx) and the net mouth opening. Abundances of sticklebacks were calculated as individuals per area sampled ($n\ m^{-2}$). A minimum of 10 sticklebacks per sampling date was frozen immediately after the catch on dry ice (−80°C) for later analyses in the laboratory. These fish were measured, weighted, dissected, sexed, and the wet stomach contents were weighed. For each sampling date, the sea surface temperature (SST) at the spawning bed was recorded.

Predator exclusion experiment—We conducted a field experiment using artificially spawned substrates to examine the effects of spawn predators on the herring egg survival and the dependence of predation intensity on the spawn concentration. To generate the sufficient amount of fertilized herring spawn for this experiment, a gillnet fishery was conducted during the spawning season (March to May 2012), targeting ripe and running herring immigrating into the study area (Fig. 1). The sticky herring eggs were carefully stripped onto clay flowerpots submerged in a bucket of seawater. The flowerpots were then transferred to a second bucket containing a mixture of seawater and herring sperm for fertilization of the eggs.

Six independently spawned flower pots were applied as artificially spawned experimental units (ASEUs) for each treatment of the predator exclusion experiment. After attaching and fertilizing herring spawn, the egg concentration on individual ASEUs was determined before the experiment, using predefined subareas at the sidewalls of the flowerpots (Fig. 2). The six subareas on every ASEU, comprising an area of 7.1 cm^2 each, were photographed, and total egg numbers were quantified using the open-source imaging software tools of ImageJ (http://imagej.nih.gov/ij). The ASEUs were transferred to a herring spawning bed in Greifswald Bay (Fig. 1) and distributed randomly within an area of ~ 25 × 25 m at a mean depth of 1.0 m. The ASEUs were installed on the seabed where different treatments were applied in terms of protection against predators: A predator exclusion (E) treatment included six ASEUs protected by a round predator exclusion cage of 65 cm in diameter equipped with netting (5 mm mesh size). The control (C) included six ASEUs that were left unprotected. Six artifact controls (AC) were installed to test for potential effects of caging on egg loss, such as, e.g., reduced effect of hydrodynamics. These controls were covered by cages with open side walls (Fig. 3). To quantify potential effects on the experimental egg numbers by natural spawning events that might occur during the investigation period, six empty flowerpots bare of herring eggs were installed additionally between the other ASEUs. These potential effects could be overlooked if no herring eggs were spawned naturally on these spawning controls during the investigation period. The experiment was run for 72 h. After exposure to the predators in the field, total egg numbers on each individual subarea of the ASEUs was determined by analyses of digital photographs similar to the examination before the experiment (Fig. 2).

Predator identification—To identify the most important herring egg predators at the study site, an additional ASEU close to the experiment was surveyed continuously using a time-lapse camera (Somikon PX-8141-919, 1.3 megapixel), taking 1 frame min^{-1} during daylight. This ASEU was installed for predator species identification only and was not included in the experimental analyses. Additionally, a

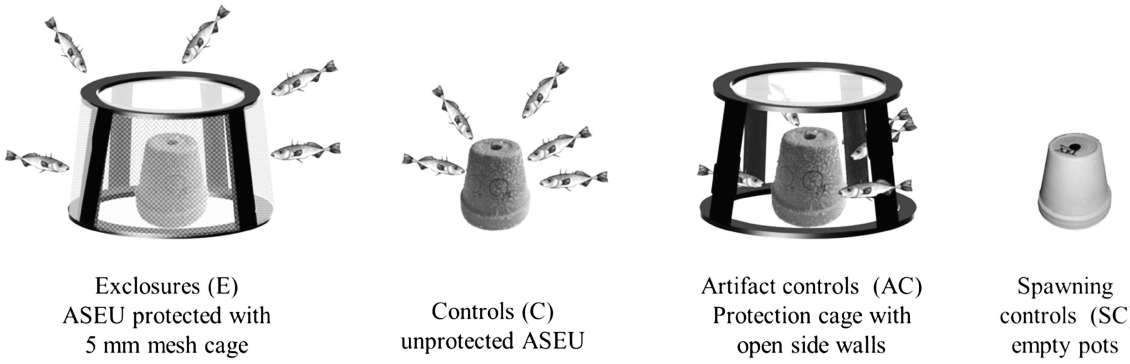

Fig. 3. Schematic illustration of the different treatments of the ASEU predator exclosure experiment. The experiment consisted of four different treatments (protected exclosures, unprotected controls, artifact controls, and natural spawning controls, six replicates each).

beach-seine (7 m opening, 5 mm mesh size) was applied to estimate predator abundance during the experiment. The different predator species were examined for basic biometric data such as total weight (TW), total length (TL), and standard length (SL; measured from snout tip to base of tail).

Feeding experiments—We performed two different feeding experiments under standardized conditions in the experimental tank facility of the Institute for Fisheries to quantify the daily consumption of herring eggs by *G. aculeatus*. Wild threespine sticklebacks were caught at a herring spawning bed within Greifswald Bay (Fig. 1) using a beach-seine (7 m opening, 5 mm mesh size). The fish were transferred to the tank facility and divided into four groups of ~ 100 individuals each. Each group was transferred to a 300 liter tank, resulting in a mean volume of 3 liters per individual. The salinity was adjusted to 6.0, corresponding to the mean spring salinity of Greifswald Bay. The fish were adapted for at least 3 d to a constant ambient temperature of 15°C in the first experiment and 11°C in the second experiment. In both experiments, the fish were starved for 3 d to ensure stomach emptiness in all individuals. Twenty fish were removed at the beginning of the experiment to estimate the stomach contents of fasting sticklebacks. The sticklebacks were then fed with fertilized herring spawn collected during the spawning season in spring. After an hour of ad libitum feeding, the remaining spawn was completely removed from the tanks to stop feeding. Five fishes were taken from each tank and immediately frozen on dry ice (−80°C), instantly interrupting the digestion process. This mode of sampling was repeated every hour until 12 h post-feeding. After 24 h, the remaining sticklebacks were removed as final samples of the experiment. For this study, the following intervals were selected for analyzing: 0 h (immediately after feeding), 4, 8, 12, and 24 h post-feeding. In the laboratory, biometric data of the fish (length, weight, sex) were recorded to characterize the experimental group composition. Stomach contents biomass (wet and dry weight) of each fish was measured for the analyses of the digestion rates.

Data analyses—For each treatment (E, C, and AC) of the predator exclusion experiment in 2012, the arithmetic mean of total egg number per ASEU was estimated at the beginning and at the end of the experiment:

$$\bar{P}_j = \left(\sum_{i=1}^{6} SA_i \right) \times n^{-1} \quad (1)$$

where $\bar{P}_j$ describes the mean total egg number per ASEU of treatment j, SA_i defines the number of eggs on ASEU-subarea i, and n the number of replicates (ASEUs) per treatment. The egg loss during the experimental period was estimated by subtracting the mean total egg number at the end of the experiment from the corresponding egg numbers prior to the experiment.

A one-way analysis of variance (ANOVA) was performed to test for the significance of the observed differences between the treatments and changes at the end of the experiment. The data were logarithmically transformed if necessary to meet the ANOVA requirement of data homoscedasticity. A Tukey's Honestly Significant Difference (HSD) multiple comparison test was performed to identify significant differences between treatments. The significance level for all statistical analyses within the study was set to $p \leq 0.05$.

Effects of egg concentration on the magnitude of mortality due to predation were analyzed exclusively focusing on egg loss in subareas of the unprotected controls (6 subareas × 6 ASEUs = n = 36). A linear regression analysis was performed to test for a relation between these two parameters. Furthermore, the relation between the relative egg loss rate (percentage of initial spawn concentration) and the initial spawn concentration was characterized with a logarithmic regression approach.

Digestion rates for both temperatures of the feeding experiment were determined using logarithmic regression analyses of the relation between stomach contents and the time post-feeding. Daily consumption rates were calculated using two different methods. We first calculated the daily consumption based on the absolute spawn digestion found 12 h after feeding. Then, mean digestion rates per hour (% h^{-1}) were used as a basis for estimates of daily consumption (mg wet weight d^{-1}). This additional calculation was conducted because it allowed a comparison of the results with related studies on digestion rates (Rajasilta 1980). The relation of herring spawn digestion by threespine

Table 1. Sea surface temperature (SST), stickleback abundance (N), number of stickleback stomachs analyzed, mean wet weight of herring spawn within the stickleback stomachs (SC_{HS} ± standard deviation [SD]), mean herring spawn concentrations ($\bar{C}_{HS}$ ± SD) at the fixed transect sampled at different calendar weeks (cw) during the spawning season in 2011. "na" indicates "not analyzed."

cw	SST (°C)	N (n m^{-2})	Stomachs (n)	SC_{HS} (g ± SD)	$\bar{C}_{HS}$ (g m^{-2} ± SD)	DP (d)	M_{PS} (%)
14	8.7	0.006	10	0.010±0.017	299.0±649.0	13	0.0002
17	10.7	0.970	29	0.068±0.063	5.4±8.9	10	11.4
19	9.8	1.057	10	0.016±0.047	0.0±0.0	11	na
21	14.3	0.913	10	0.003±0.008	5.2±10.2	7	0.5
23	17.2	1.014	0	na	0.0±0.0	6	na

stickleback and ambient temperatures was described using the Q_{10} value:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10\times(T_2-T_1)^{-1}} \quad (2)$$

The Q_{10} value is a factor describing the change of speed of biochemical reactions resulting from a temperature increase of 10 K, whereas R_1 and R_2 describe the reaction speeds at the two different temperatures, T_1 and T_2.

Spawn concentrations at the spawning bed in 2011 were extrapolated to eggs m^{-2} and dry weight m^{-2} using the arithmetic mean of six counting frame samples of each sampling date. For a comparison with data from stomach contents analyses, egg dry weight was converted to wet weight by multiplying by 9.95. This factor is based on observations by P. Kotterba (unpubl.) and represents a mean relation between wet and dry weight of herring eggs in the Greifswald Bay. The percentage of spawn that is preyed on by sticklebacks during the herring egg development period was estimated using the following equation:

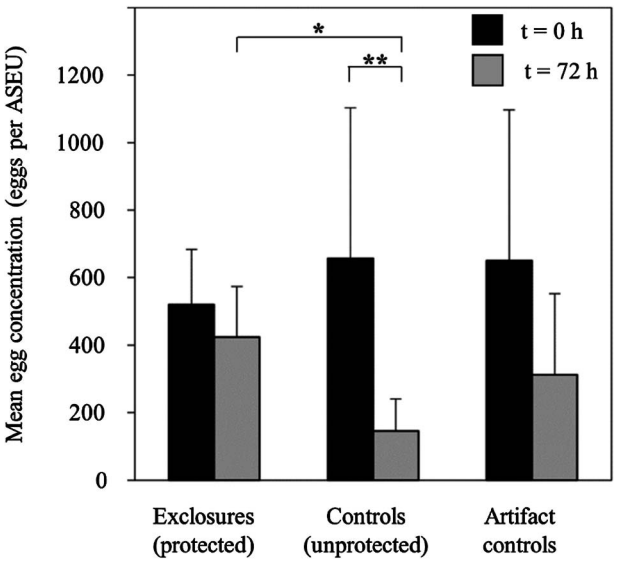

Fig. 4. Mean egg concentrations (number of eggs per ASEU) of different experimental treatments (protected exclosures, unprotected controls, and artifact control; $n = 6$) before (time, t = 0 h, black bars) and after (t = 72 h, shaded bars) the exposure to the resident estuarine predator community. Error bars represent standard deviation. Single asterisk indicates a significant difference ($p < 0.05$); double asterisks indicate a highly significant difference ($p < 0.01$).

$$M_{PS} = \frac{DC_{HS}\times N\times DP}{\bar{C}_{HS}}\times 100 \quad (3)$$

where M_{PS} describes the mortality of herring spawn due to predation by sticklebacks (%), N is the abundance of sticklebacks on the spawning ground (n m^{-2}), DP is the development time of herring eggs at a certain temperature according to Peck et al. (2012) approximated to the number of days, $\bar{C}_{HS}$ describes the mean in situ egg concentration (g wet weight m^{-2}), and DC_{HS} is defined as the mean daily herring egg consumption of a single stickleback (g wet weight per day). DC_{HS} was estimated through combining the amount of herring eggs found in the in situ stickleback stomachs with a gastric evacuation rate from the feeding experiments. For each sampling date, the gastric evacuation rate was adjusted to the corresponding SST using a rearranged Q_{10} equation. Each extrapolation was based on the assumption that SST, stickleback abundance, and individual consumption rates remain at the same level until the next sampling date.

Results

Field observations—The mean herring spawn concentrations found at the fixed transect sampled in 2011 ranged from 0 g m^{-2} dry weight in calendar weeks (cw) 19 and 23 to a maximum of 30.5 g m^{-2} in cw 14. The dry weight of herring spawn found in cw 17 (0.54 g m^{-2}) and cw 21 (0.52 g m^{-2}) was relatively low compared to the maximum at the beginning of the investigation. The extrapolated wet weights are given in Table 1. The abundance of sticklebacks was very low at cw 14 but increased by a factor of nearly 160 in cw 17 and remained at the level of ~ 1 individual m^{-2} until the end of the investigation period (Table 1).

Predator exclusion experiment—Unprotected ASEUs (C) exhibited a significant (ANOVA, $p = 0.003$; $F_{5,30} = 4.668$) reduction in herring spawn of ~ 75% after 72 h of exposure to predators in the field (Fig. 4). Tukey's HSD post hoc test showed a significant reduction of the egg concentration on the unprotected controls (Fig. 4; degrees of freedom [df] = 5; $p = 0.008$) and a significant difference between the egg concentrations on protected exclosures (E) and unprotected controls (C) after the exposure to the predators (Fig. 4; df = 5; $p = 0.029$). All other comparisons between experimental groups or points in time (before or after the exposure to predators) resulted in nonsignificant differenc-

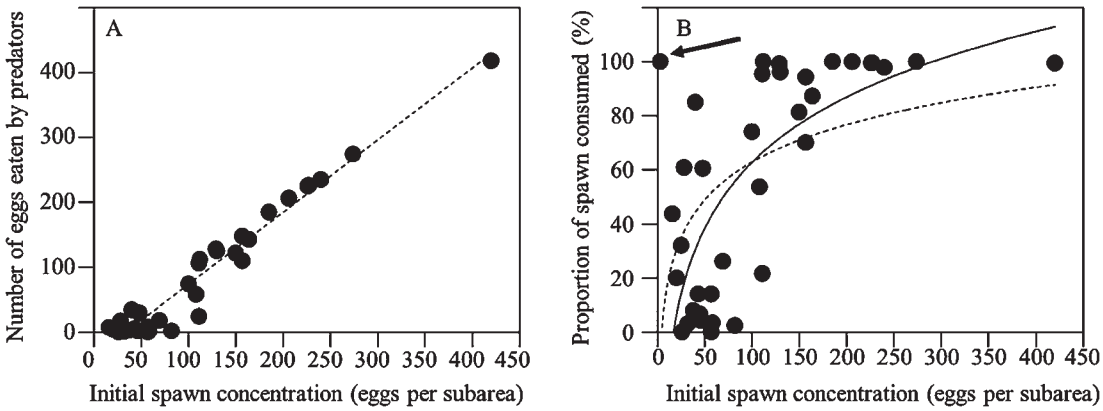

Fig. 5. Effects of egg concentration on the feeding intensity of the predators. (A) Number of eggs consumed, plotted against the initial number of eggs (dashed line = linear regression curve); (B) Proportion of herring eggs consumed (percentage of initial egg numbers) plotted against the initial number of eggs counted on these subareas. The data point marked with a black arrow is referred to as an "outlier." The dashed curve represents the logarithmic regression including the outlier, and the solid line shows the regression curve when the outlier is excluded.

es. Because no natural spawning was detected on the spawning control units after their installation, a normalization to compensate for the effects of natural spawning was not necessary.

There was a significant, linear relationship between egg concentration (eggs cm^{-2}) and the number of eggs consumed by predators (Fig. 5A; $R^2 = 0.95$; $p < 0.001$; df = 35). Plotting the relative spawn proportion removed by the predators against the initial amount of spawn resulted in a logarithmic function (Fig. 5B). When egg concentration on subareas of unprotected controls (C) was < 100 eggs (18 cases), a mean of 27% (standard deviation [SD] ± 30.7) of the eggs was consumed, whereas an egg concentration of 100 or more eggs per subarea (18 cases) induced a mean reduction of 87% (SD ± 21.0) of the initial amount of spawn. An egg number of > 180 eggs per subarea (seven cases) induced a mean reduction of 99.5% (SD ± 0.7). Logarithmic regression analyses resulted in a significant positive relationship between the initial egg concentration and the proportion consumed by the predators ($y = 19.987 \times \ln(x) - 29.25$; $R^2 = 0.244$; $p = 0.002$). This regression was stronger if a particular value was treated as an outlier: On a single subarea (Fig. 5B) only three herring eggs were found at the beginning of the experiment. Those eggs disappeared during the experiment, leading to an egg loss of 100%. If this outlier was not included in the logarithmic regression, the fitting was significantly improved ($y = 34.972 \times \ln(x) - 98.39$; $R^2 = 0.532$; $p < 0.001$).

Predator identification—We found a notable dominance of threespine stickleback among the resident estuarine fish community near the spawning bed (Table 2). Its numerical abundance is ~ 20 times higher than the second most abundant species, the ninespine stickleback (*Pungitius pungitius*). River perch (*Perca fluviatilis*) was also present, although its abundance was sevenfold lower than that of the ninespine stickleback. Camera surveillance revealed that only threespine stickleback and river perch fed intensively on the herring spawn (Fig. 6), although roach (*Rutilus rutilus*) and members of the shrimp genus *Palaemon* were also found in the beach-seine catches. Absolute abundances and biometric parameters of the examined predator species are given in Table 2.

Feeding experiments—The experiment data revealed a potentially high consumption of herring eggs by the threespine stickleback. The mean initial stomach contents directly after feeding was 62.8 mg at 11°C and 165.1 mg at 15°C. The stomach contents decreased logarithmically (Fig. 7), when plotted against the time after feeding. The logarithmic regression parameters are given in Table 3. Daily consumption was estimated based on the relative stomach contents reduction after 12 h (48% at 11°C and 76% at 15°C), resulting in daily herring egg consumption rates of 60.3 mg at 11°C and 251.0 mg at 15°C. Considering these two temperatures investigated, the increase of the digestion rate can be described by a Q_{10} value of 3.15.

Table 2. Abundance and biometrics of potential predators found in the study area during the experimental period. TL = total length, SL = standard length, TW = total weight, and SD = standard deviation. Abundances are given as numbers m^{-2} and as biomass m^{-2}. "na" indicates "not analyzed."

Species	Mean TL (mm ± SD)	Mean SL (mm ± SD)	Mean TW (g ± SD)	Abundance (n m^{-2})	Abundance (g m^{-2})
Gasterosteus aculeatus	59.6±5.0	52.9±4.1	2.1±0.6	2.978	6.879
Pungitius pungitius	45.2±4.1	40.1±3.8	0.6±0.2	0.148	0.093
Perca fluviatilis	81.3±12.2	69.3±11.5	6.1±3.0	0.028	0.173
Rutilus rutilus	na	na	na	0.014	0.078
Palaemon spp.	na	na	na	0.035	0.039

Fig. 6. Image taken by a time-lapse camera during the exclosure experiment, showing threespine stickleback and river perch feeding on herring spawn attached to an ASEU.

The dry weight of the stomach contents was 16.6% of the wet weight. Herring eggs in the stomachs remained identifiable until 8 h post-feeding, in some individual stomachs even longer. For the experiment at 11°C, a mean SL of 52.4 mm (SD ± 5 mm) was found. The sex ratio was 0.95 and the mean TW was 1.76 g (SD ± 0.4 g). The sticklebacks analyzed within the 15°C experiment had a mean SL of 53.6 mm (SD ± 5 mm) and a mean TW of 1.99 g (SD ± 0.6 g), and the sex ratio was 0.61.

Predation effects on herring spawn survival—The percentage of herring spawn potentially consumed by sticklebacks (M_{PS}) during the investigation period in 2011 is given in Table 1. No M_{PS} was calculated for cw 19 and 23 because no herring spawn was found at the spawning bed at these sampling dates.

Discussion

The general design of exclosure experiments does not allow for assignments of a predation effect to particular predator species. However, the results of the camera surveillance strongly suggested that the predation pressure caused by *G. aculeatus* was much higher than that caused by *Perca fluviatilis*. We therefore consider the threespine stickleback to be the most important piscine herring-spawn predator within Baltic Sea lagoons and estuaries. As indicated in previous studies (Winkler and Thiel 1993; Nilsson et al. 2004), *G. aculeatus* is the dominant species in the fish assemblage of many Baltic inshore systems. Its high abundance found near the area of the ASEU experiment (Table 2) is also typical for other inshore systems (Williams and Delbeek 1989; Jakobsen et al. 2003). Although *Pungitius pungitius* was found to be the second most abundant fish species on the investigated spawning bed, it was not observed feeding on herring spawn. This is in contrast to earlier studies that describe the feeding ecology of *G. aculeatus* and *P. pungitius* as being similar in terms of prey selection (Thorman and Wiederholm 1986; Hart 2003). Although our results demonstrated that predation on herring spawn is caused mainly by threespine sticklebacks, future studies should consider the potential effect of *Perca fluviatilis* because Rajasilta et al. (1993) already demonstrated that this species affects herring egg survival at the mesohaline Finnish coast.

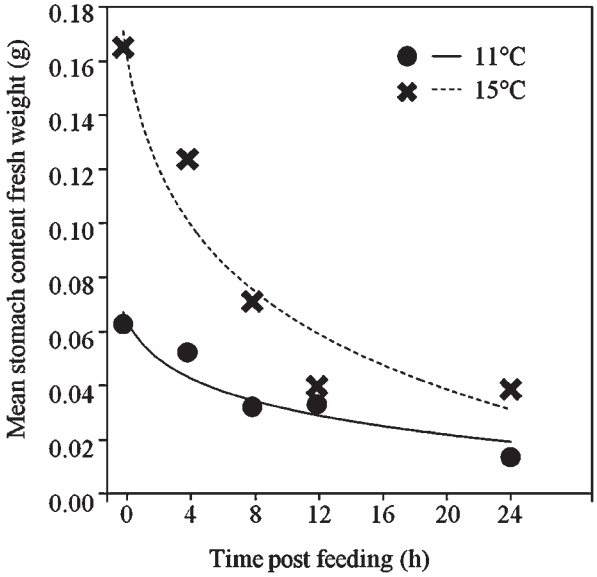

Fig. 7. Gastric evacuation of stickleback fed with herring spawn at two different ambient temperatures. Black dots and the solid regression line indicate the 11°C experiment, and black crosses and the dashed regression line indicate the 15°C experiment.

We offered herring spawn in aggregated patches to an in situ predator community and observed a significant predation effect on egg survival. Approximately 75% of the spawn on the unprotected ASEUs of the exclosure experiment was found to be already consumed after 72 h (Fig. 4). An even higher predation rate could be expected for an exposure time of 7 d, which is the mean egg development time at 15°C applied during the experiment (Klinkhardt 1986; Peck et al. 2012). However, we assume the mortality to be lower during the peak spawning period, which is characterized by lower temperatures, higher egg concentrations, and lower stickleback abundances (Table 1).

The newly established method of using artificially spawned units with standardized subareas proved to be an excellent approach for manipulative investigations with adhesive fish eggs. Artifacts caused by caging were minor and nonsignificant (Fig. 4), and the effect of the variance between the subareas was minimized by the possibility to track the fate of single subareas or even eggs.

The predation pressure on herring eggs was found depending on egg concentrations. When the relative proportion of eggs consumed was analyzed for its dependence on the initial egg concentration, an increase of egg consumption with increasing spawn concentration was observed (Fig. 5B). Beyond a threshold of ~ 180 eggs per subarea (25 eggs cm^{-2}) the predation rate reached 100% and remained at this level. We were not able to observe any inflection in the curvature because the maximum egg concentration within our experiment was apparently insufficient to satiate the large numbers of predators present. However, we assume that higher egg concentrations would lead to an overwhelming of the predator's consumption capacity and a reduction in the relative predation mortality of eggs. Nevertheless, our

Table 3. Logarithmic regression analysis of stickleback stomach contents derived from the two feeding experiments at 11°C and 15°C. Parameters (slope m, constant b, coefficient of determination R^2, and significance level p of regression) for a logarithmic gastric evacuation rate function are given for both wet and dry weights of stomach contents.

Temperature (°C)	Stomach analyses based on	Parameters of logarithmic regression with the formula $y = m \times \ln(x) + b$			
		m	b	R^2	p
11	wet weight	−0.015	0.067	0.90	0.015
	dry weight	−0.002	0.010	0.94	0.006
15	wet weight	−0.043	0.171	0.92	0.010
	dry weight	−0.005	0.023	0.72	0.071

results provide implications for estimates on in situ predation effects. The threshold described above can be extrapolated to 250,000 eggs m^{-2} or ~ 373 g m^{-2} wet weight of spawn. All mean in situ egg concentrations found in 2011 are below that value (Table 1). The highest egg concentration used on an ASEU was 420 eggs per subarea (Fig. 5) or 594,178 eggs m^{-2}. Typical egg concentrations found at peak spawning on selected herring spawning grounds of the northern hemisphere ranged between 0.001 and 7.9 million eggs m^{-2} (reviewed in Klinkhardt 1996). Further experiments using higher herring egg concentrations are needed to define the predator satiation point.

Herring eggs are mostly accumulated in patches on the spawning beds as a result of a variable distribution of spawning substrate (Scabell and Jönsson 1984). Polte and Asmus (2006) demonstrated that, in the North Sea, herring egg concentrations per plant biomass were higher on single, patchy algal stands than in extensive seagrass areas, whereas egg numbers per area were higher in the homogenous seagrass bed. Consequently, changes in the composition of spawning substrate, such as landscape partitioning, eutrophication, and fractioning of submerged vegetation, might increase the predation pressure on herring eggs by increasing the predator feeding stimulus. Predation as a driver of egg mortality should be considered acting additively to a suite of other concentration-dependent environmental factors. These factors include, e.g., oxygen depression (Klinkhardt 1996) or fungal infestations (Scabell 1988), finally affecting hatching success, especially when egg concentrations are so high as to reveal multiple egg layers.

The use of temperature-specific digestion rates is a common approach in fish biology to estimate predator- and prey-specific consumption rates (Elliott 1972). Gastric evacuation as proxy for digestion rates has been analyzed for numerous species, particularly those to be used in multispecies population-dynamic models (Temming and Herrmann 2003). As in our study, most investigations are thereby based on a single-meal approach ignoring the rather continuous feeding behavior of most predatory fish species (Bromley 1994), including threespine stickleback (Allen and Wootton 1984). However, Peck and Daewel (2007) concluded that a continuous feeding of larval and early juvenile fish might cause a 2–5-fold increase in the gastric evacuation compared to a single-meal-based feeding experiment. Since the M_{PS} values are directly proportional to the gastric evacuation rates, our single-meal approach might underestimate the predation effect.

We found a strong temperature dependence of the herring spawn consumption rates of *G. aculeatus*. The Q_{10} value for herring-spawn digestion rates based on the 12 h post-feeding values was within a range which is usual for piscine gastric evacuation processes (Temming and Herrmann 2003) but lower than the Q_{10} value of 4.29 for herring egg development, based on data provided by Klinkhardt (1986). Accordingly, lower temperatures could result in higher predation effect owing to the elongated egg phase. However, this effect is compensated by the initial amount of spawn consumed by sticklebacks at the two different temperatures. Maximum stomach filling was 2.5-fold higher at 15°C than at 11°C ambient temperature. This differs slightly from the results of Rajasilta (1980) for artificially fed fish of comparable size class, where stomach filling appeared to differ less between the distinct temperatures.

The consumption of herring eggs by threespine stickleback in our experiments exceeded the consumption of invertebrate prey offered in similar studies on *G. aculeatus* and underlined the importance of this species as herring spawn predator. Allen and Wootton (1984) found seasonally dependent in situ consumption rates between 3.5 and 19.0 mg wet weight per day. Rajasilta (1980) compared experimental feeding of *G. aculeatus* with *Daphnia* spp. in tanks with consumption rates observed in a natural environment. She found that different size classes of sticklebacks within the tanks exhibited daily consumption rates between 43.6 and 133.3 mg wet weight at an ambient temperature of 10°C, and between 49.3 and 310.7 mg at 14°C. The analysis of our data using a mean digestion rate (% h^{-1}) as described in Rajasilta (1980) revealed that individual daily consumption reaches 66.3 mg of herring spawn for 11°C and 368.7 mg for 15°C, which is clearly higher than the consumption rates found for invertebrate prey.

In our feeding experiment, we used wild threespine stickleback collected on a major herring spawning bed. Prior to the experiment, the fishes were not separated by size or sexes. Although the consumption rate is known to depend on predator body size (Temming and Herrmann 2003), the possibility of increased variability within the experimental data was accepted because the aim of the study was to characterize the natural stickleback community rather than to display an artificial composition of experimental groups.

The seasonal course of the M_{PS} (Table 1) value is mainly driven by the changes of stickleback abundances, spawn concentrations, and SST during the investigation period.

We conclude that an effect on the survival of herring eggs spawned later in the season cannot be excluded while the effect on the egg mortality during the peak spawning appears to be marginal. Nevertheless, a recent study on the survival of herring hatchlings in Greifswald Bay has shown that the recruitment success of the local herring group might be mainly determined by the number of larvae hatching during the second half of the spring spawning season (Polte et al. in press). Thus, we conclude that the threespine stickleback affects the year class survival of herring in Greifswald Bay. However, this must be interpreted with caution and further studies must address the predator–prey overlap over the entire spawning season, the effect of spawn patchiness, and the importance of distinct spawning beds for the overall recruitment success.

The coexistence of threespine stickleback and herring can be found in shallow temperate transitional waters all over the northern hemisphere. This relationship might be highlighted as an important example of the interaction between oceanic and estuarine systems. The annual amount of herring spawn deposited can reach several metric tons on a single herring spawning bed (Scabell 1988) or thousands of metric tons within a whole spawning area (Bishop and Green 2001). Regular spawning events represent an important transfer of biomass and energy from the offshore to the inshore ecosystems, which can exceed the primary production of eutrophic coastal waters during the spring peak spawning period (reviewed in Klinkhardt 1996). Compared to the entire annual primary production, this import is rather marginal but it might temporarily be important for local predators. It is widely known that such a recurring, pulsed mass availability of prey triggers the evolution of specialization in predators by simultaneously increasing the dependence on particular prey (Willson and Womble 2006). Although the transitional waters of the Baltic Sea have not been considered food limited between May and September (Thorman and Wiederholm 1986), the nutritional situation might be different during the early herring spawning period in spring (March and April) when previous to spring plankton blooms alternative prey for sticklebacks might be rare. In situ stomach contents of sticklebacks sampled in 2011 (Table 1) showed indeed a very high presence of herring eggs at certain sampling dates (e.g., 100% in cw 17; $n = 29$), indicating a directed predation on eggs. However, further investigations on the abundance, migration, and feeding behavior of threespine sticklebacks and the availability of alternative prey during the spawning season of herring is needed to determine the particular importance of this prey for the local population of *G. aculeatus*.

Improving the understanding of the herring–stickleback interaction is subject to ongoing research. A highly resolved monitoring of herring egg concentrations, seasonal SST regimes, stickleback abundances, and stomach contents will be conducted in future efforts to enhance the parameterization of our predation model.

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