Received: 14 June 2024 | Revised: 3 October 2024 | Accepted: 30 October 2024

DOI: 10.1002/ecs2.70518

ECOSPHERE
AN ESA OPEN ACCESS JOURNAL

ARTICLE

Freshwater Ecology

Operational sex ratio bias due to sex-specific cohort splitting in response to predation

Oliver Miler[1] | **František Marec**[2] | **Dietmar Straile**[1]

[1]Limnological Institute, University of Konstanz, Konstanz, Germany

[2]Biology Centre CAS, Institute of Entomology, České Budějovice, Czech Republic

Correspondence
Dietmar Straile
Email: dietmar.straile@uni-konstanz.de

Present address
Oliver Miler, Northwest Indian Fisheries Commission, 6730 Martin Way E, Olympia, Washington, USA.

Funding information
Deutsche Forschungsgemeinschaft (DFG); Collaborative Research Center (CRC), Grant/Award Number: 454

Handling Editor: Andrew M. Kramer

Abstract

The operational sex ratio (OSR), that is, the local ratio of fertilizable females to sexually active males at any given time, is of key importance for the strength of sexual selection and the reproduction of populations. We hypothesize that sex-specific cohort splitting, that is, when one sex mostly metamorphoses while the other mostly enters diapause, may lead to OSR bias in nature. The OSR of an aquatic moth, *Acentria ephemerella*, has been shown to be strongly male-biased in situ. Here, we use a mesocosm experiment in which we determine the sexes of active, diapausing, and metamorphosing larvae to test whether the male bias in *Acentria* is due to sex-specific mortality or sex-specific cohort splitting. Fish predation did not result in a strong male bias of the whole population but increased male bias in pupae and female bias in diapausing larvae. The opposite effect of fish on pupal versus diapausing larval sex ratios suggests that fish-induced sex-specific cohort splitting, rather than sex-specific mortality, caused the OSR bias of *Acentria* observed in situ. Future research needs to study whether the OSR bias is an adaptive response to the presumably higher fish predation pressure on females or a maladaptive byproduct of sex-specific activity and growth responses to fish presence. Overall, shifts in OSR due to sex-specific cohort splitting could be a more common component of arthropod life histories than previously thought.

KEYWORDS
Acentria ephemerella, aquatic insect, diapause, life history, mesocosm, metamorphosis, sex ratio, size dimorphism, stickleback predation

INTRODUCTION

Trophic interactions, such as predation and herbivory, strongly contribute to the structuring of biotic communities and ecosystems (Baxter et al., 2005; Brose et al., 2004; Peckarsky & McIntosh, 1998). Predation significantly affects the life history, behavior and population dynamics of prey organisms through direct (lethal) and indirect (nonlethal, trait-mediated) effects (Beketov & Liess, 2007; Buchanan et al., 2017; Stoks et al., 2005). Direct effects consist of predator-induced mortality, but predators can also induce a wide range of indirect effects on prey populations (Bolnick & Preisser, 2005; Lima, 1998; Relyea, 2002). Among others, these nonlethal impacts include plastic changes in growth rates, development times, feeding activity patterns and life history decisions,

such as the timing of diapause and metamorphosis (Dahl & Peckarsky, 2003; Mikolajewski et al., 2005; Stoks & Cordoba-Aguilar, 2012). The induction of nonlethal effects is especially prevalent in aquatic environments, since many aquatic organisms possess specific chemosensory structures to sense aquatic chemical stimuli from other species (kairomones) indicating the presence of predators (Crespo, 2011; Mikolajewski et al., 2005; Relyea, 2001). Predator presence, perceived through chemical and visual stimuli, has for example been shown to experimentally influence growth rates, developmental times, and size and age at metamorphosis in damselflies and mayflies (Dahl & Peckarsky, 2003; Stoks et al., 2005; Stoks & Cordoba-Aguilar, 2012).

Since generally female fecundity is strongly correlated with body mass (Garcia-Barros, 2000; Honěk, 1993), many insect species show a pronounced sexual size dimorphism with larger female than male adult body size (Mikolajewski et al., 2013; Stillwell et al., 2010; Tammaru et al., 2010). Furthermore, males and females often differ significantly in other life history and growth characteristics such as body composition and timing of metamorphosis (Chandrasegaran et al., 2018; Morbey & Ydenberg, 2001; Wiklund & Fagerström, 1977). As a result of sex-specific life history and growth patterns, nonlethal effects of predation can influence the growth patterns of both sexes differently (Chandrasegaran et al., 2018; Mikolajewski et al., 2007, 2013). Life history decisions, for example, when larvae start to metamorphose or hibernate, are often influenced by the season of the year and an organism's developmental status: A species-specific sensitive (pre-diapause) larval stage (Tauber et al., 1985) is compared to current environmental cues, resulting in the decision to either metamorphose or hibernate (Friberg et al., 2011; Gotthard, 2004; Norling, 1984). Hence, variability in developmental status may result in different life history decisions within a cohort (cohort splitting) (Crowley & Hopper, 2015; Goncalves et al., 2005; Martin et al., 1991). If male and female larvae differ in their developmental status, this may result in sex-specific cohort splitting (Denoël, Drapeau, Oromi, & Winandy, 2019; Farkas et al., 2013; Goncalves et al., 2005). As predators can affect male and female growth rates differently (Chandrasegaran et al., 2018; Mikolajewski et al., 2007, 2013), they may shift the operational sex ratio (OSR) of their prey by influencing cohort splitting differently in males and females.

Here, we study the effects of predation on sex-specific life history variability and sex-specific cohort splitting in the Lepidoptera species *Acentria ephemerella*. The life cycle of *Acentria* is predominanly aquatic with the eggs, larvae, pupae and brachypterous females remaining entirely submerged. Only the macropterous males - which are smaller than the females at metamorphosis emerge from the water (Berg, 1942; Miler et al., 2014). This size dimorphism has been shown to be accompanied in Lake Constance by a strongly male-biased pupal sex ratio, which was consistently reported for six subsequent study years (Miler et al., 2008, 2014). This sex ratio bias could be a direct effect of predation due to female larvae potentially being more vulnerable to predation because of their higher foraging activity (Brodin & Johansson, 2004; Lima & Dill, 1990; Mikolajewski et al., 2007). Alternatively, it could be an indirect effect of predation due to sex-specific cohort splitting, with female larvae preferentially interrupting their development to enter diapause, but male larvae preferentially continuing their development and metamorphosis in the presence of fish. Using a cytological method for insect larval sex determination in an ecological context (Traut et al., 1986, 2007), we determine *Acentria* sex ratios in mesocosm experiments, showing that stickleback predation influences *Acentria* sex ratio biases. Furthermore, we show that these sex ratio differences are not due to direct effects of predation, with females suffering higher mortality than males caused by, for example, accelerated growth and higher foraging rates, but rather to indirect effects leading to sex-specific cohort splitting.

MATERIALS AND METHODS

The aquatic moth *Acentria ephemerella* (Crambidae) displays a pronounced sexual wing and size dimorphism (Berg, 1942). Female pupae and adult life stages are larger than male pupae and adult life stages (Berg, 1942; Miler et al., 2014). The adult females are predominantly rudimentarily winged (brachyptery >99%), aquatic throughout their life and display morphological adaptations to the aquatic habitat such as swimming legs (Berg, 1942). Adult males, in contrast, are winged and emerge from the water column after metamorphosis (Berg, 1942). *Acentria* is a capital breeder with a short adult life span of 1–3 days, and the number of eggs can be counted in advanced pupal stages (Berg, 1942; Miler et al., 2014). Mating occurs at the water surface, with the abdominal apex of the aquatic adult females reaching above the water surface (Berg, 1942). During mating, the terrestrial males transfer a spermatophore into the bursa copulatrix of the females, which is located ventrally between the 7th and 8th abdominal segments (Berg, 1942). The larvae of *Acentria* feed from June to September on submerged aquatic macrophytes, mainly pondweed species *Potamogeton* spp. (Gross et al., 2002; Miler, 2009). A strong decrease in the biomass of its food plants occurs under high *Acentria* population densities during summer (Le Bagousse-Pinguet et al., 2012;

Miler & Straile, 2010). The life cycle of *Acentria* consists of one to three generations per year, of which one generation includes a diapause stage (Berg, 1942; Gross et al., 2002; Miler, 2009). Although information about the number of instars until metamorphosis of *Acentria* is scarce, previous research suggests five instars to be likely (Bänziger, 2000; Haenni, 1980), which fits a typical Lepidoptera life cycle with five to seven instar stages (Williams & Feltmate, 1992). Second and third-instar *Acentria* larvae of the last generation of the year enter a diapause stage in autumn, overwinter inside plant stems in a protective cocoon, the hibernaculum, and continue larval development until metamorphosis and emergence in the spring of the next year (Berg, 1942; Gross et al., 2002; Haenni, 1980; Miler et al., 2014).

The experiment was conducted in a large outdoor tank (area 52.5 m^2, depth 1.5 m) filled with fine sediment (~0.35 m) and water (~1 m) from Lake Constance (Figure 1). Fifteen mesocosms (experimental units) were placed in this tank and consisted of a transparent plastic tube (volume ~0.48 m^3, surface ~0.48 m^2, height ~1 m, length ~0.8 m, width ~0.6 m) attached to a metal frame in the sediment and to a floating frame used as a buoy to keep the plastic tube in a vertical position in the water column. Plastic tubes completely separated the experimental units from the surrounding water and there was no water exchange between the individual mesocosms and the tank. Frames were covered with metal gauze (mesh size ~1620 μm) approximately 0.1 m above the water surface, to allow for *Acentria* reproduction, but to prevent winged males from dispersing out of the replicates (for details, see Miler et al., 2008). Both the plastic tubes and the gauze-covered frames (1) prevented any *Acentria* adults or larvae from moving out of each of the mesocosms into the tank and vice versa and (2) prevented the exchange of any terrestrial organisms larger than 1620 μm in length or width and the exchange of any aquatic organisms between each of the mesocosms and the tank. Five mesocosms were sampled at the time of fish introductions (10 August, i.e., start conditions) and five experimental units were assigned to the control (no fish) and fish treatment each, and sampled on 20 August, that is, after ten days of predation. The assignment of mesocosms to treatment and time of sampling was randomized using a random number generator.

Potamogeton perfoliatus shoots were sampled on 14 June 2006 in an Upper Lake Constance macrophyte patch. Macrophyte-associated *Acentria* larvae and pupae were removed and subsequently eight macrophyte shoots were planted in each mesocosm, that is, a density of ~18 shoots m^{-2} typical for the early seasonal growing period (Wolfer & Straile, 2004). The shoots were allowed to root

FIGURE 1 Partial view of the outdoor tank with nine (most only partially visible) of altogether 15 mesocosms. The content of each mesocosm was separated from the tank water via a plastic tube and gauze-covered frames on top prevent the dispersal of flying insects (including *Acentria* adults) into and out of the mesocosms. Photo credit: Oliver Miler.

and establish new shoots for approximately five and a half weeks. From 20 to 22 July, a total of 79 *Acentria* pupae from field samples in Lower Lake Constance were introduced into each mesocosm (i.e., 1185 *Acentria* pupae in total for all 15 mesocosms taken together). Since *Acentria* larvae breathe through a thin membrane and removing this membrane would be necessary to identify the sex of the larvae (Berg, 1942), we did not know the sex of the pupae introduced into the mesocosms. Based on a field study from 2005 in Lower Lake Constance, we can assume the sex of the pupae introduced into the mesocosms was slightly male-biased (~54% males, Miler et al., 2014), whereas Upper Lake Constance sites showed a considerably higher proportion of male pupae (Miler et al., 2014). Starting on 20 July, from these pupae adult individuals hatched, mated and laid egg clutches; and on 31 July, feeding damage and small larvae could be observed in all mesocosms. Twelve sticklebacks (*Gasterosteus aculeatus*, total length range: 4.3–6.2 cm) were introduced into each mesocosm of the fish treatment on 10 August, that is, after allowing *Acentria* to develop for 10 days. In a previous mesocosm experiment at Lake Constance, a strong and significant predation of sticklebacks on *Acentria* was observed, with *Acentria* specimens found in the guts of sticklebacks (Miler et al., 2008). Sticklebacks were caught in a small pond near the University of Konstanz (geographic coordinates in decimal degrees: latitude 47.687, longitude 9.190), where they occurred in high abundances and were easy to capture with minnow traps. To our knowledge, the density of sticklebacks in Lake Constance has not been studied, but the fish density of 24 individuals m^{-2} used in our experiment was comparable to stickleback densities observed in nature (Thiel et al., 1995; Ward & FitzGerald, 1983).

Sampling occurred on 10 August to assess *Acentria* densities prior to the onset of fish predation as well as *Acentria* densities, sizes and life stage distributions on 20 August, after 10 days of fish predation and when the first adult individuals were observed. We did not observe any direct predation of *Acentria* by sticklebacks, but any such events would have been unlikely to be observed, since the mesocosms were only occasionally and briefly checked between sampling events. It is likely that active *Acentria* larvae are more vulnerable to fish predation than diapausing larvae, since the latter are better protected inside a hibernaculum and the plant stem (Berg, 1942; Gross et al., 2002; Haenni, 1980), although active *Acentria* often construct simple protective covers from pieces of leaves (Berg, 1942). We distinguished three developmental life stages of *Acentria*: active larvae (larvae outside macrophyte stems), diapausing larvae (larvae inside macrophyte stems), and pupae. *Acentria* larvae were washed through a sieve (mesh size 200 μm) and fixed in Carnoy's solution (ethanol, chloroform, acetic acid; 6:3:1) for subsequent sex determination. The remaining material washed from macrophytes, including *Acentria* larvae overlooked during the fixation in Carnoy's solution, was preserved in 70% ethanol in 100-mL plastic bottles. Since *Acentria* pupae are closely attached to the stems of *P. perfoliatus* shoots and larvae overwinter inside the stems, the remaining plant material was stored at 5°C in plastic bags and searched through for pupae and diapausing larvae within 1 week after sampling. Pupae were fixed in 70% ethanol and diapausing larvae in Carnoy's solution. Macrophytes were dried at 90°C for 3 days and densities of pupae and larvae were calculated as individuals per gram plant dry mass.

Females of most lepidopteran species of the clades Ditrysia and Tischeriina (comprising together more than 98% of all species) contain a W sex chromosome forming a densely concentrated spot (the "W-body") inside each somatic interphase nucleus, whereas males lack this feature (Figure 2a; for reviews, see Traut & Marec, 1996; Traut et al., 2007; Traut & Scholz, 1978). From each mesocosm, up to 40 active and up to 40 diapausing larvae (Appendix S1, Table 1) were dissected, and their tissue, preferentially Malpighian tubules and silk glands, was stained with 1.5% lactic acetic orcein (Traut et al., 1986). After staining, larvae were inspected for the presence of sex chromatin (W chromatin) in highly polyploid nuclei (Figure 2a) under a light microscope (Zeiss Axioskop 40) at 1000-fold magnification. In one of the five fish treatment mesocosms, the active larvae (n 11) could not be successfully sexed because these larvae were erroneously not preserved in Carnoy's solution. In addition to active and diapausing larvae from the mesocosms, we analyzed the primary sex ratio of 14 *Acentria* egg clutches that were sampled in Lake Constance and incubated at 20°C in the laboratory. From each clutch, the sex of 30 randomly selected freshly hatched larvae was determined within 24 h after hatching. *Acentria* pupae were sexed morphologically by measuring and comparing the length of wings and antennae as published in Berg (1942) (Figure 2b). To convert length measurements into dry mass, we established a head capsule width–dry mass relationship for larvae (Equation (1), n 45, R^2 0.93, $p < 0.0001$) and a length–dry mass relationship for pupae (Equation (2), n 34, R^2 0.93, $p < 0.0001$).

$$\log_{10}(\text{mass [mg]}) \quad -12.17 + (4.17 \times \log_{10}(\text{head capsule width [}\mu m\text{]})), \tag{1}$$

$$\log_{10}(\text{mass [mg]}) \quad -1.96 + (2.93 \times \log_{10}(\text{body length [mm]})). \tag{2}$$

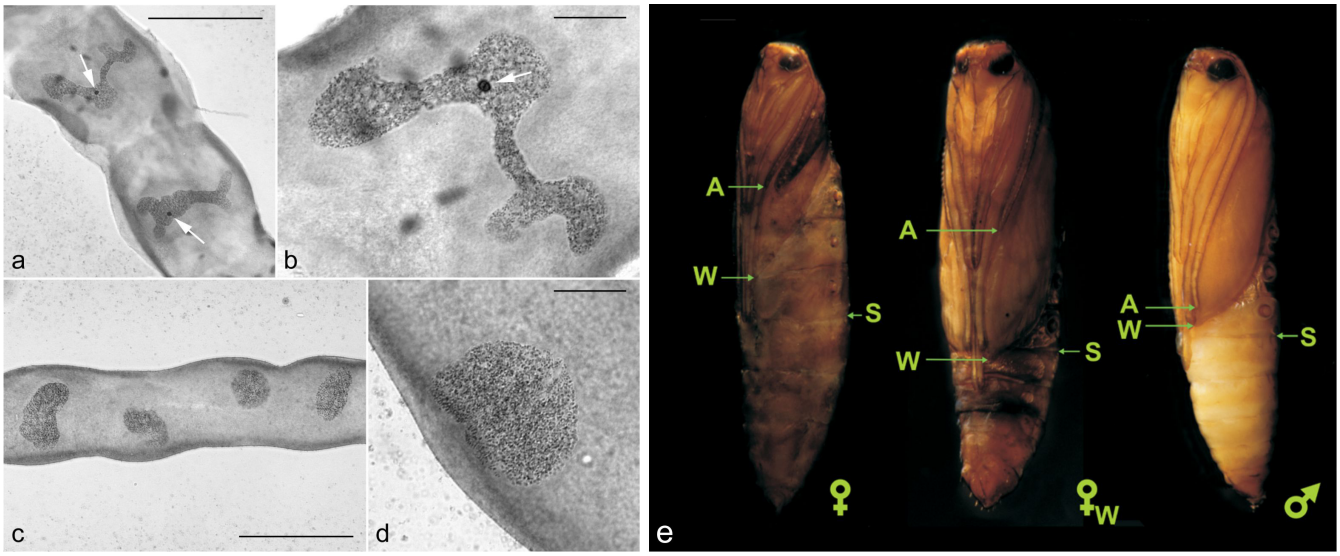

FIGURE 2 (a–d) Discrimination of *Acentria* male and female larvae by the sex chromatin body (arrows) seen in (a, b) polyploid female nuclei of the Malpighian tubules, stained with lactic acetic orcein, but (c, d) not in male nuclei. (a) A piece of the Malpighian tubule from a female larva (sex was confirmed by observation of ovaries) in the last instar with two lobed, highly polyploid nuclei. (b) A detail of a highly polyploid female nucleus. (c) A piece of the Malpighian tubule from a male larva (sex was confirmed by observation of testes) in the last instar with four spherical highly polyploid nuclei. (d) A detail of a highly polyploid male nucleus. Bar indicates (a, c) 100 μm, (b, d) 20 μm. (e) Morphological discrimination of *Acentria* male and female pupae by the length of wings (W) and antennae (A). The 2nd, 3rd, and 4th abdominal segments contain closed stigmata, and the posterior end of the 4th abdominal segment (i.e., the beginning of the 5th abdominal segment) is marked with "S." Female pupae developing into brachypterous females have short antennae and only rudimentary wing remains that do not extend over half of the length of the 5th abdominal segment (♀). Female pupae developing into macropterous females have short antennae, but wings that are longer than half of the length of the 5th abdominal segment ($♀_W$). Male pupae have always long antennae and wings extending over half of the length of the 5th abdominal segment (♂). Photo credits: Michael Korn & Oliver Miler.

TABLE 1 Densities (log_{10}-transformed) of *Acentria* as a function of the fixed factors fish presence (two levels: fish, control) and developmental stage (three levels: active larvae, diapausing larvae, pupae).

Factor	*F*	df	*p*
Developmental stage	**4.67**	**2, 16**	**0.025**
Fish presence	**9.19**	**1, 8**	**0.016**
Developmental stage × fish presence	**11.4**	**2, 16**	**<0.001**

Note: Results are from a mixed ANOVA with mesocosm ID as a random variable. Significant factors are shown in boldface.

In the following analyses, we will abbreviate the mass of larvae and pupae in log_{10}(mass [mg]) as log dm. The developmental stages of pupae were assessed based on their eye development, and eggs from specimens in an advanced developmental stage were counted after dissection.

All statistical analyses were performed with R 3.2.3 (R Development Core Team, 2015) and RStudio 0.99.489 (RStudio Inc., 2015). We used mixed-model ANOVA (package lme4, Bates et al., 2015) to study (1) the effect of fish presence and developmental stage on *Acentria* percentage densities and (2) the effect of fish presence and sex on sizes of active larvae, diapausing larvae, and pupae. The analysis of pupal sizes revealed a singularity problem for estimating the random effects. In order to confirm the results for the fixed effects, we additionally ran a simple linear model using mean sizes for each mesocosm. This only marginally altered the *F* and *p*-values. Hence, we report in the case of pupal sizes also the statistical results from the mixed-model ANOVA. The effects of fish presence on the percentage densities of active larvae, diapausing larvae, and pupae were analyzed using a multinomial generalized mixed model using a Bayesian approach (brms package, Bürkner, 2017). The effect of fish presence and developmental stage on the sex ratio was analyzed with mixed logistic regression. Type II ANOVA (*F* or Wald X^2) tests were used to test for significance of model coefficients. In all mixed models, the mesocosm ID was included as a random factor to avoid pseudoreplication. Diagnostic plots using the R package DHARMa (Hartig, 2022) suggest no violation of the assumptions for the statistical models used in this study (dispersion, homogeneity of variances). The sex ratio bias of freshly hatched larvae was analyzed with a X^2 test.

RESULTS

The density of *Acentria* at the start (10 August) of the mesocosm experiment was 37.1 ± 6.4 individuals g dm^{-1} (mean ± SE). After 10 days of predation, density had significantly declined to 9.5 ± 2.9 individuals g dm^{-1} in the fish treatment (ANOVA, sampling period; $F_{1,8}$ 13.59, p 0.006), but not in the control treatment with 48.7 ± 18.6 individuals g dm^{-1} (ANOVA, sampling period; $F_{1,8}$ 0.0012, p 0.97). Differences in densities between the fish and control treatment varied significantly for the three life stages, as indicated by the significant life stage × treatment interaction (Table 1). Densities of diapausing larvae and pupae at the end of the experiment did not differ significantly between the fish and control treatments (mixed ANOVA, treatment; diapausing larvae: $F_{1,8}$ 1.56, p 0.25; pupae: $F_{1,8}$ 2.6, p 0.15). Densities of active larvae were significantly lower in the fish treatment compared to the control treatment (mixed ANOVA, treatment; $F_{1,8}$ 25.38, p 0.001). Consequently, the posterior regression estimates of the Bayesian multinominal model of relative densities (Table 2) indicated substantially higher odds of diapausing larvae (2.21 [CrI: 1.01–3.36]) and pupae (2.29 [CrI: 1.05–3.53]) relative to active larvae in the fish treatment. Predicted probabilities showed pronounced shifts in the developmental stage composition of *Acentria* in response to fish presence (Table 3, Figure 3). Under control conditions, active larvae dominated (~55%), with lower proportions of diapausing larvae (~31%) and pupae (~14%). In the presence of fish, the relative density of active larvae declined

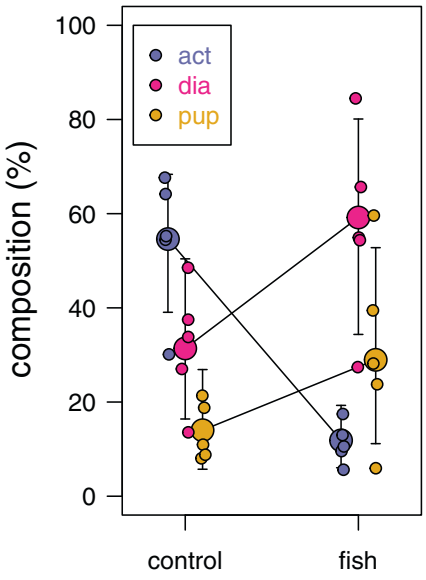

FIGURE 3 Percentage contribution of the density of active larvae (act), diapausing larvae (dia), and pupae (pup) on the overall density in the fish and control treatment at the end of the experiment. Small symbols show percentages for each mesocosm and large symbols show mean (±95% upper and lower Bayesian credible intervals) percentages for each treatment.

TABLE 2 Results of a Bayesian multinomial mixed logistic model predicting relative *Acentria* densities depending on developmental stage and fish treatment.

Stage/treatment	Estimate	Error	Lower 95% CrI	Upper 95% CrI
μ diapausing larvae intercept	−0.58	0.40	−1.37	0.22
μ pupae intercept	−1.42	0.42	−2.30	−0.59
μ diapausing larvae treatment F	2.21	0.58	1.01	3.36
μ pupae treatment F	2.29	0.62	1.05	3.53

Note: Regression coefficients, SEs, and 95% credible intervals on the log odds scale. All model parameters had Gelman-Rubin statistics $(\hat{R}) = 1$ and effective sample sizes >1000 indicating perfect convergence.

TABLE 3 Results of a Bayesian multinomial mixed logistic model predicting relative *Acentria* densities depending on developmental stage and fish treatment.

Stage	Treatment	Estimate	SE	Lower CrI	Upper CrI
Active larvae	C	0.55	0.07	0.40	0.68
Active larvae	F	0.12	0.03	0.06	0.19
Diapausing larvae	C	0.31	0.08	0.16	0.49
Diapausing larvae	F	0.59	0.12	0.34	0.79
Pupae	C	0.14	0.06	0.06	0.27
Pupae	F	0.29	0.11	0.12	0.54

strongly (~12%), whereas the proportions of diapausing larvae (~59%) and pupae (~29%) increased markedly (Table 3).

The sex ratio of *Acentria* did not significantly differ between fish and control treatments, when all life stages were taken together (% males, fish, 54.4 ± 2.3; % males, control, 48.2 ± 2.3; mixed logistic regression, factor: treatment; X^2 2.0, df 1, p 0.15; Figure 4a), and there was neither a sex ratio bias in the control nor in the fish treatment (sex ratio differences from 50%; % males, fish, t test, t 1.94, df 3, p 0.15; % males, control, t test, t −0.79, df 4, p 0.47). The presence of fish altered the sex ratios within developmental stages differently (significant developmental stage × fish interaction; Table 4 Figure 4b): The male bias of pupae was higher in the fish treatment compared to the control treatment; however, a pupal male bias was observed also in the control treatment (Figure 4b). In contrast, diapausing larvae showed a tendency for a female bias in the fish treatment (t test, t −2.48, df 4, p 0.068) and no bias in the control treatment (t test, t 0.33, df 4, p 0.76; Figure 4b). The sex ratio of freshly hatched larvae in the laboratory, that is, the primary sex ratio, showed no evidence for male bias with 50 ± 0.7 (SE) % males (X^2 0.90, df 13, p 1).

The mean size of female active larvae was lower in the fish compared to the control treatment, whereas for male active larvae no difference between the two treatments was observed (significant interaction between sex and fish presence; Figure 5a, Table 5). Female diapausing larvae were significantly larger than male diapausing larvae, and there was a tendency for diapausing larvae of both sexes in the fish treatment to be larger than diapausing larvae in the control treatment (Figure 5b, Table 5). Female pupae were significantly larger than male pupae, and both sexes were significantly smaller in the presence of fish (Figure 5c, Table 5). Female pupal size was tightly linked to clutch size (adjusted R^2 0.82, $p < 0.001$; Figure 6). A reduction of mean female pupal size from 0.81 ± 0.016 log dm in the fish treatment to 0.71 ± 0.017 log dm in the control as observed in this experiment was

TABLE 4 *Acentria* sex ratio as a function of the fixed factors fish presence (two levels: fish, control) and developmental stage (three levels: active larvae, diapausing larvae, and pupae).

Factor	X^2	df	p
Developmental stage	**38.8**	**2**	**<0.001**
Fish presence	1.76	1	0.18
Developmental stage × fish presence	**6.19**	**2**	**<0.05**

Note: Results of a mixed logistic regression with mesocosm ID as a random variable are shown, with significant factors in boldface.

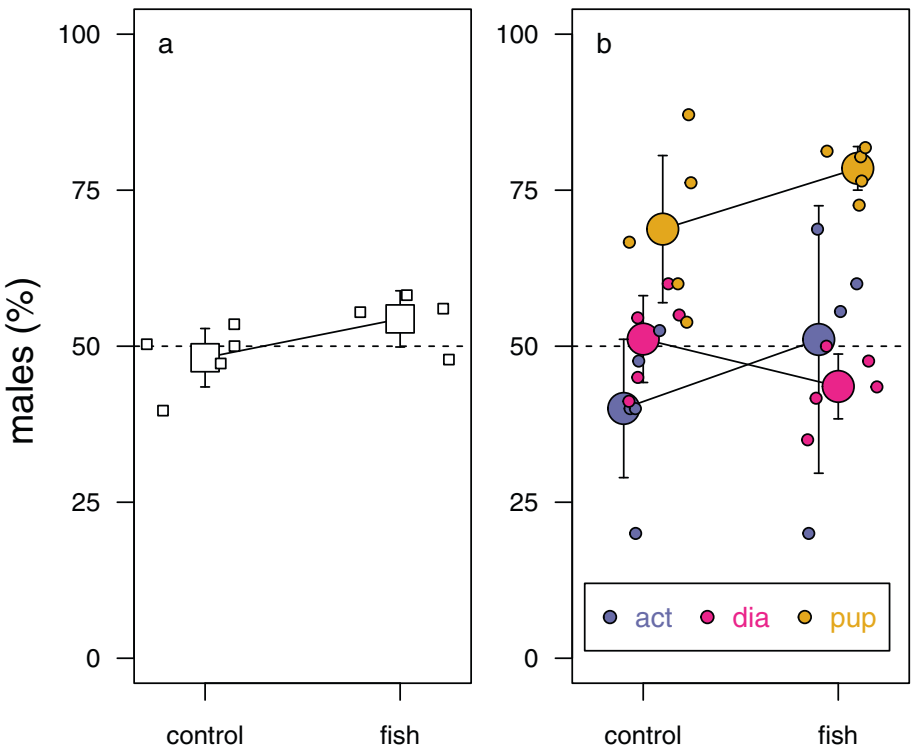

FIGURE 4 Percentage of males in the fish and control treatment of (a) all life stages combined and (b) separately for active larvae (act), diapausing larvae (dia), and pupae (pup). Small symbols show percentages for each mesocosm, and large symbols show mean (±2 SE) percentages for each treatment.

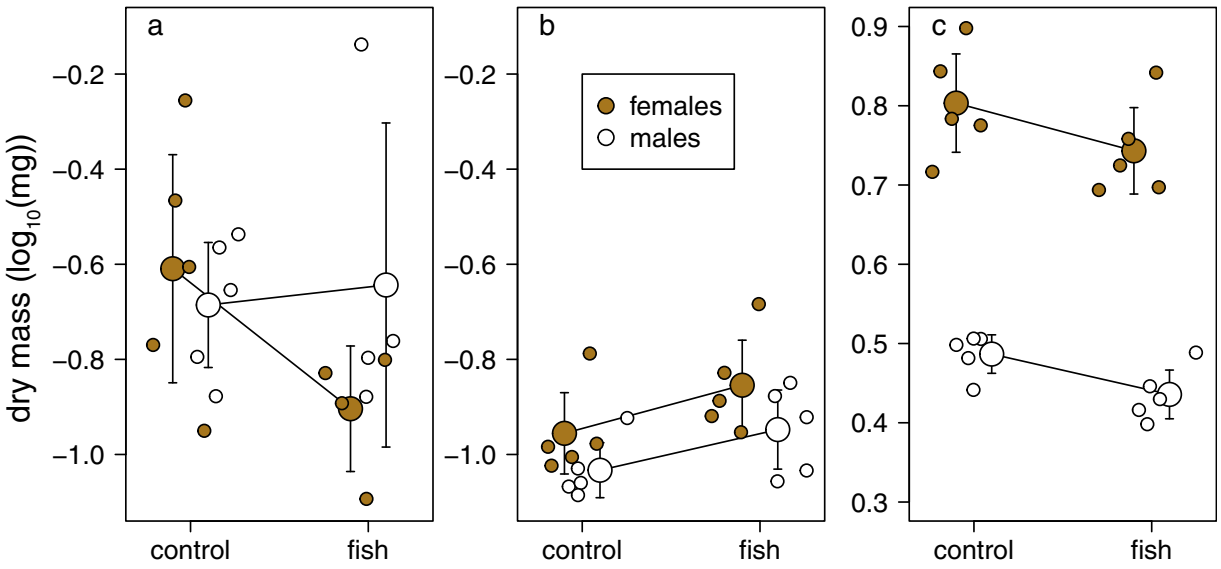

FIGURE 5 Mean male and female dry masses for (a) active larvae, (b) diapausing larvae, and (c) pupae in control and fish treatments. Small symbols show mean dry masses for each mesocosm and large symbols show mean (±2 SE) dry masses for each treatment. Note the scale difference of the *y*-axis for panel (c).

TABLE 5 Body sizes (log dm) of *Acentria* active larvae, diapausing larvae, and pupae as functions of the fixed factors fish presence (2 levels: fish, control) and sex (2 levels: males, females).

Factor	*F*	df	*p*
Size of active larvae			
Sex	0.94	1, 7	0.33
Fish presence	0.83	1, 7	0.36
Sex × fish presence	**4.91**	1, 7	**0.027**
Size of diapausing larvae			
Sex	**11.44**	**1, 8**	**<0.001**
Fish presence	3.25	1, 8	0.07
Sex × fish presence	0.097	1, 8	0.76
Size of pupae			
Sex	**186.42**	**1, 8**	**<0.001**
Fish presence	**5.91**	**1, 8**	**0.015**
Sex × fish presence	0.042	1, 8	0.84

Note: Results of a mixed ANOVA with mesocosm ID as a random variable are shown, with significant factors in boldface.

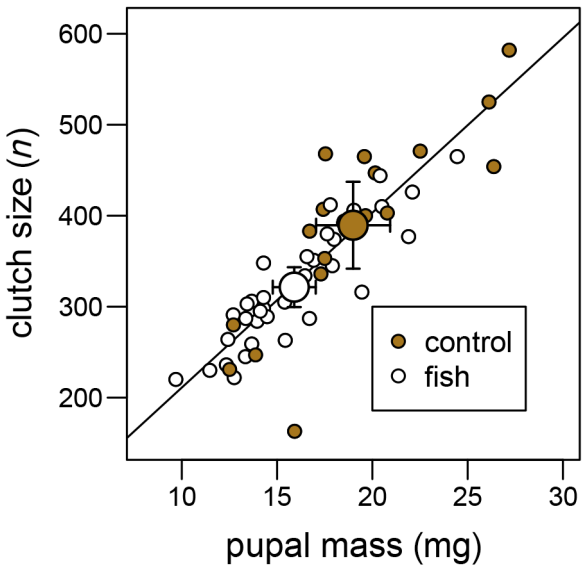

FIGURE 6 Relationship between pupal mass and number of eggs in the fish treatment and control. Small circles show data for individual pupae, whereas large circles show the mean (±2 SE) values in the fish treatment and control, respectively.

equivalent to a reduction of the clutch size by approximately 19% (from 329.3 ± 10.9 to 266.8 ± 11.4 eggs) (mixed ANOVA, treatment; $F_{1,8}$ 5.44, p 0.020).

DISCUSSION

Stickleback predation strongly reduced total *Acentria* densities and additionally had a strong effect on several life history characteristics: life stage distributions, life stage specific sex ratios, sizes at metamorphosis of female and male individuals, and clutch sizes. Using sex chromatin staining of polyploid nuclei from larvae, we found strong evidence that the observed bias in field OSRs (pupal sex ratios, Miler et al., 2008; Miler et al., 2014) is likely not a direct effect of predation but is best explained by sex-specific life history decisions of larvae in the presence of predators.

It could also be argued, however, that sex-specific mortality due to fish predation might result in a strongly male-biased pupal sex ratio and a weakly male-biased sex ratio of all life stages combined. This would require that the sex-specific mortality is low for small larvae and increases strongly with larval size. Stomach content analyses of sticklebacks though did not detect a preference of sticklebacks for large larvae, suggesting that small larvae are also exposed to predation pressure (Miler et al., 2008). A higher mortality of females would also not be an explanation for the observed significantly higher female bias for diapausing larvae in the fish treatment compared to the control. Since the female diapausing larvae were larger than the male diapausing larvae, small female larvae are also likely to be more active than small male larvae. We would expect this to result in higher female mortality in small larvae and consequently a stronger male bias in diapausing larvae in the fish treatment compared to the control, that is, opposite to the observed pattern. In contrast to sex-specific mortality, sex-specific cohort splitting can explain the observed biases for all stages without the strong assumption that sex-specific mortality strongly increases with larval size. This suggests that sex-specific mortality alone is not a sufficient mechanism to explain the sex ratio biases observed in our experiment and thus also the strongly male-biased OSR repeatedly observed in situ (Miler et al., 2008, 2014).

When interpreting the pupal and diapausing larval sex ratios, it must be taken into account that the observed sex ratios were still transient, as a large percentage of *Acentria* were still in the active larval stage, especially in the control mesocosms (~55%), but not in the fish mesocosms (~10%). Hence, these larvae would still have to either diapause or metamorphose. This explains why the pupal sex ratio bias in the control mesocosms was also rather high (68% males, Figure 4b). As the mean size of the active larvae in the control mesocosms was already larger than the mean size of diapausing larvae (Figure 5), there is a high probability that most of these larvae would actually have metamorphosed. The metamorphosis of these larvae, whose sex ratio was female-biased (Figure 4b), will reduce the pupal sex ratio bias in the control mesocosms. If only 40% of all active larvae would pupate, this would reduce the percentage of males in the pupal stage from $68.8\% \pm 5.9\%$ to $49.9\% \pm 3.9\%$ in the control treatment. In contrast, in the fish mesocosms, the same percentage of active larvae metamorphosing would not strongly modify the pupal sex ratio (from $78.5\% \pm 1.7\%$ to $74.2\% \pm 2.2\%$), as in these mesocosms the number of active larvae was much lower. Hence, the subsequent development of larvae would ultimately have resulted in an unbiased OSR in the control, but a highly male-biased OSR in the fish treatment.

The decision of arthropod larvae to either enter diapause, that is, to interrupt larval development until metamorphosis, or to metamorphose depends on the sensitive larval stage (a specific pre-diapause developmental stage in which diapause-inducing stimuli are perceived, e.g., in the egg, larval, prepupal, or pupal stage; Tauber et al., 1985) relative to the current environmental cues. For example, at a given photoperiod, larvae less developed than a specific developmental stage (instar number) or smaller than a specific size will diapause, whereas larvae with a size/developmental stage beyond that size/developmental stage threshold will metamorphose (Lindestad et al., 2021; Norling, 1984; Numata & Shintani, 2023). If larvae smaller and larger than the size/developmental stage threshold are present concurrently, this will result in cohort splitting via midpoint cueing (Crowley & Hopper, 2015; Kozáčeková et al., 2009; Norling, 1984). Individuals in a stage more and less advanced than this threshold will follow a fast (developmental stage > sensitive stage) and slow (developmental stage ≤ sensitive stage) developmental trajectory, respectively.

In *Acentria*, the sensitivity of the developmental stages and consequently the developmental pathways is likely sex-specific in response to predation, resulting in males and females developing via fast track (metamorphosis) or slow track (diapause) pathways. Such sex-specific effects of predation have recently been demonstrated in amphibians (newts), where more males choose the progenetic over the metamorphic developmental pathway due to higher fitness and reproductive activity costs compared to females (Denoël, Drapeau, & Winandy, 2019). In addition, sex-specific differences in developmental pathways have been found in newts in response to climatic drivers (temperature) and termed the "male escape hypothesis" (Mathiron et al., 2017).

In *Acentria*, diapausing female larvae are larger than diapausing male larvae, suggesting that females need to grow faster than males in order to pass the size/developmental stage threshold for development to metamorphosis. Female larvae of various arthropod species achieve a larger size at metamorphosis compared to males by higher growth rates (Inkpen & Foellmer, 2010; Stillwell et al., 2010; Vendl et al., 2018). These higher growth rates can be achieved by higher feeding activities or foraging more in riskier habitats, which would make them more vulnerable to predators (Brodin & Johansson, 2004; Peckarsky et al., 1993; Werner & Anholt, 1993). Consequently, a stronger reduction of female growth rates relative to male growth rates in response to predators would result in more female larvae entering diapause as compared to males, and more males than females metamorphosing. This reasoning suggests that the

observed bias in *Acentria* OSR may be a side product of developmental decisions involved in cohort splitting. Although the effect of the fish treatment on the size of diapausing larvae was not significant (p 0.07), there was a tendency for male and female diapausing larvae to be larger in the fish treatment than in the control (Table 5, Figure 5b). This result seems counterintuitive given that we could expect a slower growth of larvae in the presence of fish compared to the control treatment, which has been shown in other studies (Brodin & Johansson, 2004; Martin et al., 1991). A possible reason for this result might be that fish presence may also cause larger larvae to diapause, that is, those larvae which might have already reached a comparatively large size when fish were introduced.

An important question for subsequent studies would be to answer whether the sex-specific life history decisions resulting in OSR bias of *Acentria* are adaptive or nonadaptive. A low number of reproducing females could have a strong negative influence on population growth rates, and a low number of diapausing males could impair population development in the next season, which might suggest that the observed sex ratio bias is maladaptive. However, the observed life history decisions of male and female larvae might also be adaptive due to possibly larger fitness costs of a smaller size at metamorphosis for females (Garcia-Barros, 2000; Honěk, 1993; Miler et al., 2014) compared to males. Hence, in order to avoid the costs of a small size at metamorphosis, females might opt for diapause and to complete their development in the next season. Furthermore, the decision for diapause might be adaptive for females since after metamorphosis females might experience a larger predation pressure from fish relative to males. While male imagos develop wings after eclosion and remain above the water surface, most females develop only rudimentary wings and remain in the water (Berg, 1942). Consequently, fish predation pressure on adult females might be larger than on adult males, especially in high summer, when large numbers of young-of-the-year sticklebacks are present: The reproduction of sticklebacks in the preceding spring period (Hyatt & Ringler, 1989; Poizat et al., 2002) suggests that juveniles contribute to an increasing stickleback density in macrophyte patches towards summer. However, further work is needed to quantify whether and under what conditions the benefits of sex-specific life cycle decisions outweigh the presumed costs of biased OSRs.

To conclude, we could reject our hypothesis that male bias is a direct predation effect and found strong support for our alternative hypothesis that sex-specific life cycle decisions of moth larvae result in the highly male-biased pupal sex ratios in the field. This highlights the importance of studying arthropod life cycle strategies in a sex-specific manner, which requires the sex determination of larvae either via cytological (Aron et al., 2003; Traut et al., 2007; Traut & Marec, 1996) or genomic methods (Ali et al., 2019; Belousova et al., 2019; Fuková et al., 2009). As cohort splitting (Crowley & Hopper, 2015; Goncalves et al., 2005; Martin et al., 1991) and sexual size dimorphism (Blanckenhorn et al., 2007; Stillwell et al., 2010; Tammaru et al., 2010) are widespread characteristics of arthropod life histories, further studies examining their combined effects on OSR are warranted.

ACKNOWLEDGMENTS

We thank Gisela Richter for the processing of the plant material and Martin Wolf for help with the experimental setup of the mesocosm experiment. Karl-Otto Rothhaupt and Karl Gotthard provided helpful comments on a previous version of the manuscript, and Luca Schenone advised us in using the Bayesian multinominal model. We thank three anonymous reviewers for their helpful and constructive comments. A number of students assisted in running the experiment and analysis of samples: Robin Assfalg, Konrad Bergen, Christoph Berron, Anke Dopychai, and Stefanie Eschenbächer. Elisabeth Gross provided advice on the experimental use of *A. ephemerella* and *P. perfoliatus*. This research project was part of the Collaborative Research Center (CRC) number 454 "Littoral of Lake Constance" and was financially supported by the Deutsche Forschungsgemeinschaft (DFG). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (Miler et al., 2025) are available from Dryad: https://doi.org/10.5061/dryad.8kprr4xtg.

ORCID

Oliver Miler https://orcid.org/0000-0002-4781-244X
František Marec https://orcid.org/0000-0002-6745-5603
Dietmar Straile https://orcid.org/0000-0002-7441-8552

REFERENCES

Ali, B., Y. Zhou, Q. Zhang, C. Niu, and Z. Zhu. 2019. "Development of an Easy and Cost-Effective Method for Non-invasive Genotyping of Insects." *PLoS One* 14: e0216998.

Aron, S., L. de Menten, and D. van Bockstaele. 2003. "Brood Sex Ratio Determination by Flow Cytometry in Ants." *Molecular Ecology Notes* 3: 471–75.

Bänziger, R. 2000. "Spatio-Temporal Distribution of Size Classes and Larval Instars of Aquatic Insects (Ephemeroptera, Trichoptera and Lepidoptera) in a *Potamogeton pectinatus* L. Bed (Lake Geneva, Switzerland)." *Revue Suisse de Zoologie* 107: 139–151.

Bates, D. M., M. Mächler, B. M. Bolker, and S. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67: 1–48.

Baxter, C. V., K. D. Fausch, and W. C. Saunders. 2005. "Tangled Webs: Reciprocal Flows of Invertebrate Prey Link Streams and Riparian Zones." *Freshwater Biology* 50: 201–220.

Beketov, M. A., and M. Liess. 2007. "Predation Risk Perception and Food Scarcity Induce Alterations of Life-Cycle Traits of the Mosquito *Culex pipiens*." *Ecological Entomology* 32: 405–410.

Belousova, I., N. Ershov, S. Pavlushin, Y. Ilinsky, and V. Martemyanov. 2019. "Molecular Sexing of Lepidoptera." *Journal of Insect Physiology* 114: 53–56.

Berg, K. 1942. "Contributions to the Biology of the Aquatic Moth *Acentropus niveus* (Olivier)." *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening* 105: 59–139.

Blanckenhorn, W. U., A. F. G. Dixon, D. J. Fairbairn, M. W. Foellmer, P. Gibert, K. van der Linde, R. Meier, et al. 2007. "Proximate Causes of Rensch's Rule: Does Sexual Size Dimorphism in Arthropods Result from Sex Differences in Development Time?" *The American Naturalist* 169: 245–257.

Bolnick, D. I., and E. L. Preisser. 2005. "Resource Competition Modifies the Strength of Trait Mediated Predator–Prey Interactions: A Meta-Analysis." *Ecology* 86: 2771–79.

Brodin, T., and F. Johansson. 2004. "Conflicting Selection Pressures on the Growth/Predation-Risk Trade-Off in a Damselfly." *Ecology* 85: 2927–32.

Brose, U., A. Ostling, K. Harrison, and N. D. Martinez. 2004. "Unified Spatial Scaling of Species and Their Trophic Interactions." *Nature* 428: 167–171.

Buchanan, A. L., S. L. Hermann, M. Lund, and Z. Szendrei. 2017. "A Meta-Analysis of Non-consumptive Predator Effects in Arthropods: The Influence of Organismal and Environmental Characteristics." *Oikos* 126: 1233–40.

Bürkner, P.-C. 2017. "Brms: An R Package for Bayesian Multilevel Models Using Stan." *Journal of Statistical Software* 80: 1–28.

Chandrasegaran, K., S. R. Kandregula, S. Quader, and S. A. Juliano. 2018. "Context-Dependent Interactive Effects of Non-lethal Predation on Larvae Impact Adult Longevity and Body Composition." *PLoS One* 13: e0192104.

Crespo, J. G. 2011. "A Review of Chemosensation and Related Behavior in Aquatic Insects." *Journal of Insect Science* 11: 1–39.

Crowley, P. H., and K. R. Hopper. 2015. "Mechanisms for Adaptive Cohort Splitting." *Ecological Modelling* 308: 1–13.

Dahl, J., and B. L. Peckarsky. 2003. "Developmental Responses to Predation Risk in Morphologically Defended Mayflies." *Oecologia* 137: 188–194.

Denoël, M., L. Drapeau, N. Oromi, and L. Winandy. 2019. "The Role of Predation Risk in Metamorphosis Versus Behavioural Avoidance: A Sex-Specific Study in a Facultative Paedomorphic Amphibian." *Oecologia* 189: 637–645.

Denoël, M., L. Drapeau, and L. Winandy. 2019. "Reproductive Fitness Consequences of Progenesis: Sex-Specific Pay-Offs in Safe and Risky Environments." *Journal of Evolutionary Biology* 32: 629–637.

Farkas, A., T. Jakab, O. Müller, A. Móra, I. Lajter, and G. Dévai. 2013. "Sex Ratio in Gomphidae (Odonata) at Emergence: Is there a Relationship with Water Temperature?" *International Journal of Odonatology* 16: 279–287.

Friberg, M., I. M. Aalberg Haugen, J. Dahlerus, K. Gotthard, and C. Wiklund. 2011. "Asymmetric Life-History Decision-Making in Butterfly Larvae." *Oecologia* 165: 301–310.

Fuková, I., L. G. Neven, N. M. Bárcenas, N. A. Gund, M. Dalíková, and F. Marec. 2009. "Rapid Assessment of the Sex of Codling Moth *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) Eggs and Larvae." *Journal of Applied Entomology* 133: 249–261.

Garcia-Barros, E. 2000. "Body Size, Egg Size, and their Interspecific Relationships with Ecological and Life History Traits in Butterflies (Lepidoptera: Papilionoidea, Hesperioidea)." *Biological Journal of the Linnean Society* 70: 251–284.

Goncalves, S. C., M. A. Pardal, P. G. Cardoso, S. M. Ferreira, and J. C. Marques. 2005. "Biology, Population Dynamics and Secondary Production of *Tylos europaeus* (Isopoda, Tylidae) on the Western Coast of Portugal." *Marine Biology* 147: 631–641.

Gotthard, K. 2004. "Growth Strategies and Optimal Body Size in Temperate Parnassiinae Butterflies." *Integrative and Comparative Biology* 44: 471–79.

Gross, E. M., C. Feldbaum, and C. Choi. 2002. "High Abundance of Herbivorous Lepidoptera Larvae (*Acentria ephemerella* DENIS & SCHIFFERMULLER) on Submersed Macrophytes in Lake Constance (Germany)." *Fundamental and Applied Limnology* 155: 1–21.

Haenni, J.-P. 1980. "Contribution à la connaissance de la biologie des papillons aquatiques (Lepidoptera, Pyraloidea) sur la rive sud du lac de Neuchâtel." *Bulletin de la Société Neuchâteloise des Sciences Naturelles* 103: 29–43.

Hartig, F. 2022. "DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models." R Package Version 0.4.6

Honěk, A. 1993. "Intraspecific Variation in Body Size and Fecundity in Insects: A General Relationship." *Oikos* 66: 483–492.

Hyatt, K. D., and N. H. Ringler. 1989. "Role of Nest Raiding and Egg Predation in Regulating Population Density of Threespine Sticklebacks (*Gasterosteus aculeatus*) in a Coastal British Columbia Lake." *Canadian Journal of Fisheries and Aquatic Sciences* 46: 372–383.

Inkpen, S. A., and M. W. Foellmer. 2010. "Sex-Specific Foraging Behaviours and Growth Rates in Juveniles Contribute to the Development of Extreme Sexual Size Dimorphism in a Spider." *The Open Ecology Journal* 3: 59–70.

Kozáčeková, Z., J. M. T. de Figueroa, M. J. López-Rodríguez, P. Beracko, and T. Derka. 2009. "Life History of a Population of *Protonemura intricata* (RIS, 1902) (Insecta, Plecoptera) in a Constant Temperature Stream in Central Europe." *International Review of Hydrobiology* 94: 57–66.

le Bagousse-Pinguet, Y., E. M. Gross, and D. Straile. 2012. "Release from Competition and Protection Determine the Outcome of Plant Interactions along a Grazing Gradient." *Oikos* 121: 95–101.

Lima, S. L. 1998. "Nonlethal Effects in the Ecology of Predator-Prey Interactions." *Bioscience* 48: 25–34.

Lima, S. L., and L. M. Dill. 1990. "Behavioral Decisions Made under the Risk of Predation: A Review and Prospectus." *Canadian Journal of Zoology* 68: 619–640.

Lindestad, O., I. M. Aalberg Haugen, and K. Gotthard. 2021. "Watching the Days Go by: Asymmetric Regulation of Caterpillar Development by Changes in Photoperiod." *Ecology and Evolution* 11: 5402–12.

Martin, T. H., D. M. Johnson, and R. D. Moore. 1991. "Fish-Mediated Alternative Life-History Strategies in the Dragonfly *Epitheca cynosura*." *Journal of the North American Benthological Society* 10: 271–79.

Mathiron, A. G. E., J.-P. Lena, S. Baouch, and M. Denoël. 2017. "The 'Male Escape Hypothesis': Sex-Biased Metamorphosis in Response to Climatic Drivers in a Facultatively Paedomorphic Amphibian." *Proceedings of the Royal Society B: Biological Sciences* 284: 20170176.

Mikolajewski, D. J., T. Brodin, F. Johansson, and G. Joop. 2005. "Phenotypic Plasticity in Gender Specific Life-History: Effect of Food Availability and Predation." *Oikos* 110: 91–100.

Mikolajewski, D. J., G. Joop, and B. Wohlfahrt. 2007. "Coping with Predators and Food Limitation: Testing Life History Theory for Sex-Specific Larval Development." *Oikos* 116: 642–49.

Mikolajewski, D. J., B. Wohlfahrt, G. Joop, and A. P. Beckerman. 2013. "Sexual Size Dimorphism and the Integration of Phenotypically Plastic Traits." *Ecological Entomology* 38: 418–428.

Miler, O. 2009. *The Aquatic Moth Acentria ephemerella as a Key Species in Submerged Aquatic Vegetation – Direct and Trait-Mediated Interactions with Predators and Food Plants*. Konstanz, Germany: University of Konstanz.

Miler, O., E. M. Gross, and D. Straile. 2014. "Small-Scale Variation in Sexual Size Dimorphism and Sex Ratio in the Aquatic Moth *Acentria ephemerella* Denis and Schiffermüller, 1775 (Lepidoptera: Crambidae)." *Aquatic Insects* 36: 187–199.

Miler, O., M. Korn, and D. Straile. 2008. "Experimental Evidence for a Strong Influence of Stickleback Predation on the Population Dynamics and Sex Ratio of an Aquatic Moth." *Fundamental and Applied Limnology* 173: 187–196.

Miler, O., F. Marec, and D. Straile. 2025. "Operational Sex Ratio Bias Due to Sex-Specific Cohort Splitting in Response to Predation [Dataset]." Dryad. https://doi.org/10.5061/dryad.8kprr4xtg.

Miler, O., and D. Straile. 2010. "How to Cope with a Superior Enemy? Plant Defence Strategies in Response to Annual Herbivore Outbreaks." *Journal of Ecology* 98: 900–907.

Morbey, Y. E., and R. C. Ydenberg. 2001. "Protandrous Arrival Timing to Breeding Areas: A Review." *Ecology Letters* 4: 663–673.

Norling, U. 1984. "Life History Patterns in the Northern Expansion of Dragonflies." *Advances in Odonatology* 1: 127–156.

Numata, H., and Y. Shintani. 2023. "Diapause in Univoltine and Semivoltine Life Cycles." *Annual Review of Entomology* 68: 257–276.

Peckarsky, B. L., C. A. Cowan, M. A. Penton, and C. R. Anderson. 1993. "Sublethal Consequences of Stream-Dwelling Predatory Stoneflies on Mayfly Growth and Fecundity." *Ecology* 74: 1836–46.

Peckarsky, B. L., and A. R. McIntosh. 1998. "Fitness and Community Consequences of Avoiding Multiple Predators." *Oecologia* 113: 565–576.

Poizat, G., E. Rosecchi, and A. J. Crivelli. 2002. "Life-History Variation within a Three-Spined Stickleback Population in the Camargue." *Journal of Fish Biology* 60: 1296–1307.

R Development Core Team. 2015. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Relyea, R. A. 2001. "Morphological and Behavioral Plasticity of Larval Anurans in Response to Different Predators." *Ecology* 82: 523–540.

Relyea, R. A. 2002. "The Many Faces of Predation: How Induction, Selection, and Thinning Combine to Alter Prey Phenotypes." *Ecology* 83: 1953–64.

RStudio Inc. 2015. *RStudio: Integrated Development Environment for R*. Boston, MA: RStudio Inc.

Stillwell, R. C., W. U. Blanckenhorn, T. Teder, G. Davidowitz, and C. W. Fox. 2010. "Sex Differences in Phenotypic Plasticity Affect Variation in Sexual Size Dimorphism in Insects: From Physiology to Evolution." *Annual Review of Entomology* 55: 227–245.

Stoks, R., and A. Cordoba-Aguilar. 2012. "Evolutionary Ecology of Odonata: A Complex Life Cycle Perspective." *Annual Review of Entomology* 57: 249–265.

Stoks, R., M. de Block, F. van de Meutter, and F. Johansson. 2005. "Predation Cost of Rapid Growth: Behavioural Coupling and Physiological Decoupling." *Journal of Animal Ecology* 74: 708–715.

Tammaru, T., T. Esperk, V. Ivanov, and T. Teder. 2010. "Proximate Sources of Sexual Size Dimorphism in Insects: Locating Constraints on Larval Growth Schedules." *Evolutionary Ecology* 24: 161–175.

Tauber, M. J., C. A. Tauber, and S. Masaki. 1985. *Seasonal Adaptations of Insects*. Oxford, UK: Oxford University Press.

Thiel, R., A. Sepulveda, R. Kafemann, and W. Nellen. 1995. "Environmental Factors as Forces Structuring the Fish Community of the Elbe Estuary." *Journal of Fish Biology* 46: 47–69.

Traut, W., and F. Marec. 1996. "Sex Chromatin in Lepidoptera." *The Quarterly Review of Biology* 71: 239–256.

Traut, W., K. Sahara, and F. Marec. 2007. "Sex Chromosomes and Sex Determination in Lepidoptera." *Sexual Development* 1: 332–346.

Traut, W., and D. Scholz. 1978. "Structure, Replication and Transcriptional Activity of the Sex-Specific Heterochromatin in a Moth." *Experimental Cell Research* 113: 85–94.

Traut, W., A. Weith, and G. Traut. 1986. "Structural Mutants of the W Chromosome in *Ephestia* (Insecta, Lepidoptera)." *Genetica* 70: 69–79.

Vendl, T., P. Šípek, O. Kouklík, and L. Kratochvíl. 2018. "Hidden Complexity in the Ontogeny of Sexual Size Dimorphism in Male-Larger Beetles." *Scientific Reports* 8: 5871.

Ward, G., and G. J. FitzGerald. 1983. "Fish Predation on the Macrobenthos of Tidal Salt Marsh Pools." *Canadian Journal of Zoology* 61: 1358–61.

Werner, E. E., and B. R. Anholt. 1993. "Ecological Consequences of the Trade-Off between Growth and Mortality Rates Mediated by Foraging Activity." *American Naturalist* 142: 242–272.

Wiklund, C., and T. Fagerström. 1977. "Why Do Males Emerge Before Females?" *Oecologia* 31: 153–58.

Williams, D. D., and B. W. Feltmate. 1992. *Aquatic Insects*. Wallingford, Oxfordshire: CAB International.

Wolfer, S. R., and D. Straile. 2004. "Spatio-Temporal Dynamics and Plasticity of Clonal Architecture in *Potamogeton perfoliatus*." *Aquatic Botany* 78: 307–318.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Miler, Oliver, František Marec, and Dietmar Straile. 2026. "Operational Sex Ratio Bias Due to Sex-Specific Cohort Splitting in Response to Predation." *Ecosphere* 17(1): e70518. https://doi.org/10.1002/ecs2.70518