Foraging behaviour, swimming performance and malformations of early stages of commercially important fishes under ocean acidification and warming

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Abstract

Early life stages of many marine organisms are being challenged by climate change, but little is known about their capacity to tolerate future ocean conditions. Here we investigated a comprehensive set of biological responses of larvae of two commercially important teleost fishes, *Sparus aurata* (gilthead seabream) and *Argyrosomus regius* (meagre), after exposure to future predictions of ocean warming (+4 ºC) and acidification (ΔpH=0.5). The combined effect of warming and hypercapnia elicited a decrease in the hatching success (by 26.4 and 14.3% for *S. aurata* and *A. regius*, respectively) and larval survival (by half) in both species. The length for newly-hatched larvae was not significantly affected, but a significant effect of hypercapnia was found on larval growth. However, while *S. aurata* growth was reduced (24.8-36.4% lower), *A. regius* growth slightly increased (3.2-12.9% higher) under such condition. Under acidification, larvae of both species spent less time swimming, and displayed reduced attack and capture rates of prey. The impact of warming on these behavioural traits was opposite but less evident. While not studied in *A. regius*, the incidence of body malformations in *S. aurata* larvae increased significantly (more than tripled) under warmer and hypercapnic conditions. These morphological impairments and behavioural changes are expected to affect larval performance and recruitment success, and further influence the abundance of fish stocks and the population structure of these commercially important fish species. However, given the pace of ocean climate change, it is important not to forget that species may have the opportunity to acclimate and adapt.

Keywords: Ocean climate change, fish early stages, survival and growth, malformations, behaviour, ecophysiology

Introduction

Atmospheric CO_2 levels are rising at an unprecedented rate. The continuous absorption of atmospheric CO_2 by oceans is causing a decline in ocean's pH, which is expected to decrease 0.4-0.5 units by the year 2100. In parallel, sea surface temperature is expected to rise by up to 4 °C by the end of the century (Collins et al. 2013). Ocean warming and acidification represent a major threat to many marine organisms by affecting their acid-base balance, metabolism, growth and behaviour (Munday et al. 2011; Pörtner et al. 2004) in ways that often compromise species fitness and survival (Wittmann and Pörtner 2013).

Fishes were thought to be quite resilient to exposure to elevated CO_2, given their strong ability to regulate acid-base balance by bicarbonate accumulation and ion exchange across the gills (Melzner et al. 2009). Nevertheless, fish early life stages have shown to be more susceptible to elevated CO_2 than adult fish (reviewed by Pörtner et al. 2005). Several studies have reported direct effects of elevated pCO_2 on survival, growth, metabolism, behaviour, otoliths and skeletal development of marine fish larvae (Baumann et al. 2012; Frommel et al. 2014; Munday et al. 2011; Pimentel et al. 2014). Other studies have found no significant effects of increasing pCO_2 on fish larvae (Harvey et al. 2013; Hurst et al. 2013; Maneja et al. 2013b), suggesting species-specific responses to changing ocean conditions.

To date, very few studies have investigated the susceptibility of early stages of commercially important fish species to climate-driven changes, including codfish (Frommel et al. 2014) and yellowfin tuna (Bromhead et al. 2015). Given the importance of larval growth and survival rates to the year-class success in marine fish populations (Peck et al. 2012), deleterious effects of climate-driven changes in pCO_2 and temperature may have profound consequences on the distribution and abundance of marine fish stocks (Pörtner and Peck 2010).

Here we analysed the effects of ocean warming (+4 ºC) and acidification (ΔpH=0.5) on the development and behaviour of early life stages of two commercially important fish species in the NE Atlantic Ocean, namely the gilthead seabream *Sparus aurata* and the meagre *Argyrosomus regius*.

Methods

Egg collection

Eggs of *S. aurata* were collected at the hatchery Maresa (Mariscos de Estero, Spain), in November 2013. Eggs of *A. regius* were obtained from Instituto Português do Mar e da Atmosfera (IPMA) - Centro Regional de Investigação Pesqueira do Sul (CRIPSul, Olhão, Portugal), in May 2014. Meagre eggs were obtained from a wild-caught broodstock of four females and two males. Eggs were collected immediately after spawning and transferred to the aquaculture facilities in Laboratório Marítimo da Guia (Cascais, Portugal). At approximately 5 hours after spawning, eggs were acclimated to the different experimental conditions.

Egg incubation and larval rearing

Following an acclimation period of about 2 h, *S. aurata* and *A. regius* eggs were exposed to four experimental treatments, a cross-factor design of two temperatures and two pCO_2 levels: **(1)** control temperature and normocapnia (pCO_2=~350 µatm, pH=8.0); **(2)** control temperature and hypercapnia (pCO_2=~1400 µatm, pH=7.5, ΔpH=0.5); **(3)** the expected warming scenario (+4 °C) and normocapnia; and **(4)** warming and hypercapnia. Control temperatures represented the average temperature during the spawning season of *S. aurata* (18 °C; Arias 1980) and *A. regius* (20 °C; Quéro and Vayne 1987).

For each species and treatment, eggs and larvae were reared in 3 independent recirculating systems (12 per species in total), each composed of a 19 L cylindrical rearing tank connected to a 100 L sump. To ensure an accurate water temperature in each experimental treatment, the rearing tanks were placed inside 400-L water bath tanks. All rearing systems were filled with filtered (1 µm) and UV-irradiated seawater (salinity 35). Temperatures were kept stable via seawater chiller systems. pH levels were monitored with glass pH probes (Schott®Instruments) connected to a ProfiLux system (GHL, Germany). pH was automatically adjusted via solenoid valves, by injecting a certified CO_2 gas mixture into the water or by aerating the water with CO_2 filtered air (by using CO_2 scrubbers with soda lime). Salinity, temperature and pH levels were also manually monitored daily. Total

alkalinity was spectrophometrically determined (at 595nm) according to Sarazin et al. (1999). pH and alkalinity measurements were used for pCO_2 calculation (see Supplementary Table 1 for seawater carbonate chemistry), using the CO2SYS program (Lewis and Wallace, 1998), with dissociation constants from Mehrbach et al. (1973) as refitted by Dickson and Millero (1987). Ammonia and nitrites were monitored regularly and maintained within recommended levels.

For the embryonic development experiment, 10 eggs were randomly placed inside a small rearing box in each rearing tank, and followed for approximately 43 hours until hatching. The remaining eggs were distributed in egg-incubation tanks and further used for the larval experiment. After hatching, larvae were carefully counted and transferred to the rearing tanks. *S. aurata* larvae were randomly distributed at a density of 70 larvae L^{-1} and reared until 15 days post-hatch (dph). Larvae were fed on rotifers (*Brachionus plicatilis*) at an increasing density of 5 to 10 rot mL^{-1} between 2 and 15 dph, and *Artemia* nauplii (0.2-2 art mL^{-1}) from 10 to 15 dph [adapted from Fernández et al. (2008)]. *A. regius* larvae were reared at a density of 45 larvae L^{-1} for 10 days. Larvae started to feed on rotifers (from 5 to 10 rot mL^{-1}) between 2 and 10 dph, and *Artemia* nauplii (0.2-2 art mL^{-1}) was gradually introduced at 6 dph until the end of the experiment [based on Pousão-Ferreira et al. (2013)]. Both rotifers and *Artemia* nauplii were enriched with Red Pepper. At the end of each day, prey availability in each rearing tank was checked to ensure that prey density was never a limiting factor regardless of the treatment. The light regime in both experiments was 14 L:10 D.

Hatching success, survival and growth

The hatching success and larval survival were determined per rearing tank, based on the number of surviving larvae at hatching and at the end of the experiment, respectively. In each tank, the standard length at hatching and at the end of the experiment (i.e., 15 dph and 10 dph for *S. aurata* and *A. regius*, respectively) was measured for 4 individuals (12 per treatment) using a dissecting microscope. The somatic length growth (SLG) was calculated as the difference between the mean length at hatching and the length of each larva at the end of the experiment divided by the time elapsed.

Body malformations

At the end of the experiment, 20 *S. aurata* larvae per tank (60 per treatment) were sampled and fixed in 4% buffered paraformaldehyde for 24 h, and then transferred to 70% ethanol. Larvae were observed under a microscope to identify and quantify body structure malformations and/or axial deviations, based on Boglione et al. (2001). Malformations were classified according to the affected area (cranium, abdominal and caudal region). Cranium malformations included asymmetric eye, deformed meckel's cartilage in the jaw and deformed ceratobranchial in the opercle. Abdominal and caudal malformations included abnormal body curvatures such as side-to-side, excessive inward and outward curvatures, and abnormal urostyle flexion. Malformations were quantified as the percentage of fish exhibiting a specific deformity. The incidence of body malformations in *A. regius* larvae was not assessed.

Behavioural patterns

Behavioural observations of *S. aurata* and *A. regius* larvae were conducted at the end of both experiments. A preliminary study was performed to establish the ethogram for both species (Table 2). Swimming (S) and spin (Sp) behaviours were recorded as time variables, whereas miss (M), attack (A) and capture (C) behaviours were recorded as frequency variables. The capture success was calculated as the fraction of successful attack events (based on Drost 1987). The behavioural patterns were analysed through direct observation by a "blind observer", using the focal animal technique. Four larvae per rearing tank (12 per treatment) were randomly selected and the behaviour of each larva was analysed inside the rearing tanks during 1 minute, 30 min after feeding.

Statistical analyses

Experiments led to data expressed as (1) proportions (hatching success, survival success, and malformations), (2) counts (number of observed behaviours), and (3)

positive quantities (measures of lengths and growths). All data were analysed via generalized linear mixed models (GLMM, e.g. Zuur et al. 2009). The distributional family considered was binomial (logit link function), Poisson (logit link function) and Gaussian (identity link function) for proportions, counts and positive quantities, respectively. Model's residuals were checked for departures from the assumed distributions and no significant deviations were found. The mixed model component was introduced to respect the properties of the experimental design, i.e., box/tank were always included as a random effect to account for possible dependency within tanks. Following the recommendation from Barr et al. (2013), we kept the random effects in the model irrespectively of the amount of variation it explained. All models considered included the same 2-level fixed effects, i.e., temperature and pH, as well as their all second order interaction. For body malformations, behaviour and survival, which response could be conditional on the rearing time, we did not include species as an explanatory variable in the model to avoid confounding between effects by species and rearing time. For variables not dependent on rearing time, we considered species as an additional fixed effect, as well as the corresponding three second order interactions. The most parsimonious models were selected based on Akaike Information Criterion and used for inference. This potentially allowed to borrow strength across species to find significant treatment effects. Odds ratios and confidence limits are also presented for Binomial models, allowing a more informative discussion of the results. Odds were defined as the ratio of the probability of success and the probability of failure, and odds ratios were build by the ratio of odds under control temperature or normocapnia and odds under warming or hypercapnia. All statistical analyses were implemented in R (R Core Team, 2014), using the hglm package (Ronnegard et al. 2010).

Results

Hatching success and survival

Regarding hatching success (Fig. 1A,B), the most parsimonious model included the main effects of temperature (β=-0.721, SE=0.272, p=0.008) and pCO_2 (β=-0.599,

SE=0.271, p=0.027). Neither the effect of species (p=0.579) nor the interaction between both factors (p=0.981) was significant. The odds of hatching under warming were only 0.39 (95% CI: 0.29, 0.76) times the odds of hatching under control temperature, i.e., the odds ratio of hatching were approximately 2.6 higher at control temperature. The odds of hatching under hypercapnia were only 0.59 (95% CI: 0.32, 1.15) times the odds of hatching under normocapnia i.e., the odds ratio of hatching were approximately 1.69 higher under normocapnia.. Specifically, the hatching success of *S. aurata* decreased from 88.3±7.6% in the control to 65.0±10.0% in the future scenario, while the hatching success of *A. regius* decreased from 93.3±5.7% in the control to 80.0±10.0% in the future scenario.

In terms of survival, a model was considered for each species. Regarding *S. aurata* (Fig. 1C), the main effects of temperature (β=-1.237, SE=0.130, p<0.001) and pCO_2 (β=-0.313, SE=0.130, p=0.016) were significant, with survival being lower under higher temperature and hypercapnia. The odds of survival under warming were only 0.29 (95% CI: 0.22, 0.37) times the odds of survival under control temperature, i.e., the odds ratio of survival was approximately 3.5 higher at control temperature. The odds of survival under hypercapnia were 0.73 (95% CI: 0.57, 0.94) times the odds of survival under normocapnia, i.e., the odds ratio of survival was approximately 1.4 higher at normocapnia. However, the interaction between temperature and pCO_2 was not significant (p=0.414). Survival rates of this species decreased from 43.3±2.8% under control conditions to 20.8±2.9% under the future scenario. Regarding *A. regius* (Fig. 1D), the main effects of temperature (β=-1.015, SE=0.113, p<0.001) and pCO_2 (β=-0.301, SE=0.113, p=0.008) were significant, but the interaction between them was not (p=0.236). The odds of survival under warming were only 0.36 (95% CI: 0.29, 0.45) times the odds of survival under control temperature, i.e., the odds ratio of survival was 2.8 higher at control temperature. The odds of survival under hypercapnia were 0.74 (95% CI: 0.59, 0.92) times the odds of survival under normocapnia, i.e., the odds ratio of survival was 1.4 higher at normocapnia. The survival of this species decreased from 40.0±10.0% under control to 20.0±5.0% under future conditions.

Length and growth

The mean standard length of newly-hatched larvae (at 0 dph) was independent of the treatment (Fig. 2A,B), with no terms found to be significant (p=0.284, p=0.982 and p=0.393 for temperature, pCO_2 and the interaction between both factors, respectively). Size at hatching ranged between 2.6±0.2 and 2.7±0.3 mm for *S. aurata*, and between 2.5±0.5 and 2.7±0.3 mm for *A. regius*. Considering growth (Fig. 2C,D), the main effect of pCO_2 was significant (β=-0.028, SE=0.008, p=0.009), as well as the interaction between pCO_2 and species (β=0.037, SE=0.012, p=0.003). However, neither temperature (p=0.098) nor species (p=0.107) had a significant effect. SLG was higher for *A. regius*, with values ranging from 0.09 to 0.11 mm day^{-1}, while for *S. aurata* it ranged from 0.06 to 0.09 mm day^{-1}. The significant interaction between pCO_2 and species arises from the fact that growth was higher under normocapnia for *S. aurata* but higher under hypercapnia for *A. regius*.

Body malformations

Malformations were only assessed for *S. aurata* (Figs. 3 and 4). Regarding total malformations (Fig. 4A), both the main effects temperature (β=0.946, SE=0.328, p=0.004) and pCO_2 (β=1.730, SE=0.332, p<0.01) were significant, but the interaction between both factors was not (p=0.287). The odds of total malformations under warming were 2.57 (95% CI: 1.35, 4.90) times the odds of malformations under control temperature, while the odds under hypercapnia were 5.64 (95% CI: 2.95, 10.80) times the odds under normocapnia. The incidence of malformations increased from 25.00±13.23% under control conditions to 81.67±10.41% under the warmer and acidified scenario. The only term included in the cranium malformations model was pCO_2 (β=1.704 SE=4.272, p<0.001), with a higher proportion of malformations observed under hypercapnia (Fig. 4B). Neither temperature (p=0.729) nor the interaction between both factors (p=0.166) was significant. The odds under hypercapnia were 5.50 (95% CI: 2.52, 12.02) times the odds under normocapnia. Under elevated pCO_2, the incidence of cranium malformations increased significantly from 8.33±2.88 to 21.67±2.87% under control temperature, and from 6.67±2.89 to 40.00±5.00% under warming. With respect to abdominal malformations (Fig. 4C), none of the variables was significant (p=0.232, p=0.232 and p=0.539 for temperature, pCO_2 and the interaction between

both factors, respectively). For caudal malformations (Fig. 4D), there was not enough information to build a model, since this type of malformation was only observed in one of the four treatments. However, a great proportion (26.7±2.9%) of the fish in the warmer and acidified scenario presented this malformation.

Behavioural patterns

In terms of behaviour (Fig. 5), a model was considered for each species. For *S. aurata*, swimming (Fig. 5A) was only significantly affected by pCO_2 (β=-18.333, SE=2.590, p<0.001), and not by temperature (p=0.164). Additionally, no significant interaction was found between both factors (p=0.243). Swimming duration significantly decreased with pCO_2 from 47.6±6.3 to 32.5±6.1 sec at normal temperature, and from 53.2±12.4 to 31.6±9.4 sec at the warming condition. For attack (Fig. 5C), both temperature (β=0.352, SE=0.163, p=0.031) and pCO_2 (β=-1.214, SE=0.261, p<0.001) were found significant, as well as the corresponding interaction (β=0.862, SE=0.308, p=0.005). More attacks happened under higher temperatures, but this increase was more pronounced under hypercapnia than normocapnia. The capture success (Fig. 5E) was also significantly affected by pCO_2 (β=-1.466, SE=0.320, p=0.000), but not by temperature (p=0.145). Moreover, the interaction between both factors (β=0.802, SE=0.382, p=0.036) was also significant. The capture success of this species decreased significantly under hypercapnic conditions, from 4.3±1.7 to 1.0±0.4% under control temperature, and from 5.7±1.4 to 3.0±0.9% at warmer temperatures. For spin behaviour (Fig. 5G), there was not enough information to build the model because this behaviour was only observed under warming and hypercapnic conditions.

Regarding *A. regius*, swimming (Fig. 5B) was significantly affected by temperature (β=8.750, SE=1.817, p=0.001) and pCO_2 (β=-10.917, SE=1.817, p=0.000), but no significant interaction was found between factors (p=0.627). Warmer temperature significantly increased the time larvae spent swimming from 40.1±5.6 to 49.7±4.4 sec under normocapnia, and from 30.1±5.8 to 37.9±5.1 sec under hypercapnia. In contrast, swimming duration significantly decreased with pCO_2, from 40.1±5.6 to 30.1±5.8 sec at normal temperature, and from 49.7±4.4 to 37.9±5.1 sec at the warming condition. The attack (Fig. 5D) and capture rates (Fig. 5F) were only

significantly affected by pCO_2 (β=-0.367, SE=0.146, p=0.012 and β=-0.693, SE=0.158, p<0.001, respectively). Neither the effect of temperature (p=0.250) nor the interaction (p=0.459) was significant. Attack rates decreased significantly with pCO_2 from 5.3±2.7 to 1.6±0.9% at control temperature, and from 7.6±2.1 to 5.3±2.0% at warming. The capture success also decreased significantly with acidification from 4.8±2.7 to 2.1±0.8% under present-day temperature, and from 5.2±2.4 to 2.9±1.4% under warming. No spin behaviour was observed for this species (Fig. 5H).

Discussion

In the present study, we showed that early life stages of *S. aurata* and *A. regius* were quite sensitive to future ocean conditions. Both warming and acidification significantly lowered the hatching and survival rates of both species. In comparison to the present day scenario, the survival of 15 dph seabream and 10 dph meagre decreased by half under future warming and acidified conditions. The standard length of newly-hatched larvae was not significantly affected, but hypercapnia had a significant effect on larval growth. However, while the SLG of *S. aurata* showed a 24.8-36.4% decrease under hypercapnic conditions, a slight increase (3.2-12.9%) was observed in *A. regius*. The former results may suggest a weak control and maintenance of internal pH on *S. aurata* and a consequent decrease in protein biosynthesis (Langenbuch and Pörtner 2003). In this species, the energy budget may have been allocated away from non-essential processes, such as growth, towards maintenance (Pörtner and Peck 2010). The present difference observed between species reinforces the absence of consensus among studies on the effect of ocean acidification on the size and growth of marine fish larvae. While some studies have reported decreased size and growth under high pCO_2 levels (Baumann et al. 2012; Frommel et al. 2014; Pimentel et al. 2014), others indicate that larvae may grow equally well or even faster under high pCO_2 conditions (Hurst et al. 2013; Hurst et al. 2012; Schade et al. 2014). If the impact on growth is truly species-specific, then ocean acidification and warming may have a complex impact on the dynamics of marine food webs, since larval growth and body size can mediate susceptibility to predation mortality (Anderson 1988).

Nonetheless, we can also argue that such contradictory findings may be the result of experiments being carried out at temperatures with unclear positioning on thermal performance curves, or possibly due to experimental rearing artefacts (e.g. different feeding regimes). It is worth noting that our findings on survival and growth under present-day conditions were quite similar to those found in the literature for these species under intensive rearing conditions (Papandroulakis et al. 2000; Roo et al. 2010).

Ocean warming and acidification also had a significant effect on the incidence of malformations, with the exception of abdominal malformations. At present-day conditions, the formation pattern of the axial skeleton elements in *S. aurata* was similar to that reported for other teleost larvae (Sfakianakis et al. 2004). Under the combined effect of hypercapnia and warming, the incidence of malformations greatly increased. The incidence of total malformations was more than 3 times higher under future warming and acidified conditions than when compared to present-day conditions. Cranium malformations also increased significantly with ocean acidification. Under the future scenario, the occurrence of this malformation was 31.7 percentage points higher than in present-day conditions. Although there was not enough information to build a model for caudal axial deviations, it has to be noticed that almost 30% of the fish presented this malformation when exposed to the combined effect of warming and acidification. Other studies also found greater incidence of abnormal development in fish larvae under elevated temperature and/or pCO_2 (Baumann et al. 2012; Pimentel et al. 2014; Ahnelt et al. 2015). During early development, axial deviations may result from defective development of the notochord and perinotochordal connective sheet (Sanatamaría et al. 2005), which can in turn lead to further skeletal malformations in the vertebral column such as lordosis, scoliosis and kyphosis. We argue that such malformations may affect the larval capacity to maintain the position in the water column, and further compromise their swimming, foraging and predator avoidance (Powell et al. 2009).

In the present study, larval behaviour was affected by future ocean conditions. Temperature increased the time *A. regius* larvae spent swimming, but did not affect *S. aurata* swimming. In contrast, hypercapnia decreased the time spent swimming in both species. Interestingly, *S. aurata* larvae showed an erratic “spin” movement

only at higher temperatures and pCO_2 levels. Even though some previous studies have found no effect of ocean acidification on fish swimming behaviour (e.g. Maneja et al. 2013a), others have reported significant changes in this behaviour under such environmental conditions (e.g. Dixson et al. 2010; Munday et al. 2010). Reduced swimming skills and the occurrence of erratic movements by the larvae in the wild may potentially affect their vulnerability to predation. Moreover, hypercapnia also decreased the attack and capture rates of prey. The lower capture success of prey will most certainly impact their growth and development, and further affect larval performance, survival and recruitment rates (Stanley 2009). It is however important to keep in mind that some bias may potentially arise from the effects that ocean changes might have on live prey and larvae-prey interaction, which directly affects food availability and fish larval behaviour.

In conclusion, the biological responses of *S. aurata* and *A. regius* larvae presented in the present study provide an insight of how future warming and acidification may impact the development of wild fish larvae and their fitness in a changing ocean. However, results should be carefully interpreted, given the reduced genetic variability that may arise from a limited number of spawners. Moreover, given the time frame in which ocean warming and acidification are expected to occur, it is important not to forget that there will be an opportunity for acclimatization and adaptation. Although the mechanisms for adaptation remain poorly known, some studies have already shown that parental (transgenerational) acclimation can modify the response of fish larvae to climate change conditions (e.g. Schade et al. 2014; Welch et al. 2014). It is therefore expected that such processes can moderate the negative impacts of future ocean conditions on *S. aurata* and *A. regius* larvae. Future efforts should focus on how these environmental factors may affect commercially important fish species at higher levels of organization (e.g. at a population-level) in a way to help managers and policy-makers to take proactive measures.

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Author contributions

R.R. and M.S.P. designed the experiment; M.S.P. and G.D. performed the experiments; M.S.P., F.F., T.M., R.B., G.D., J.M., P.P.F. and R.R. analysed the data; M.S.P., F.F., T.M., R.B., G.D., A.M.F., J.M., M.P., H.P., E.J.G. and R.R. wrote the main paper. All authors discussed the results and their implications, and commented on the manuscript at all stages.

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Figure

Figure 1

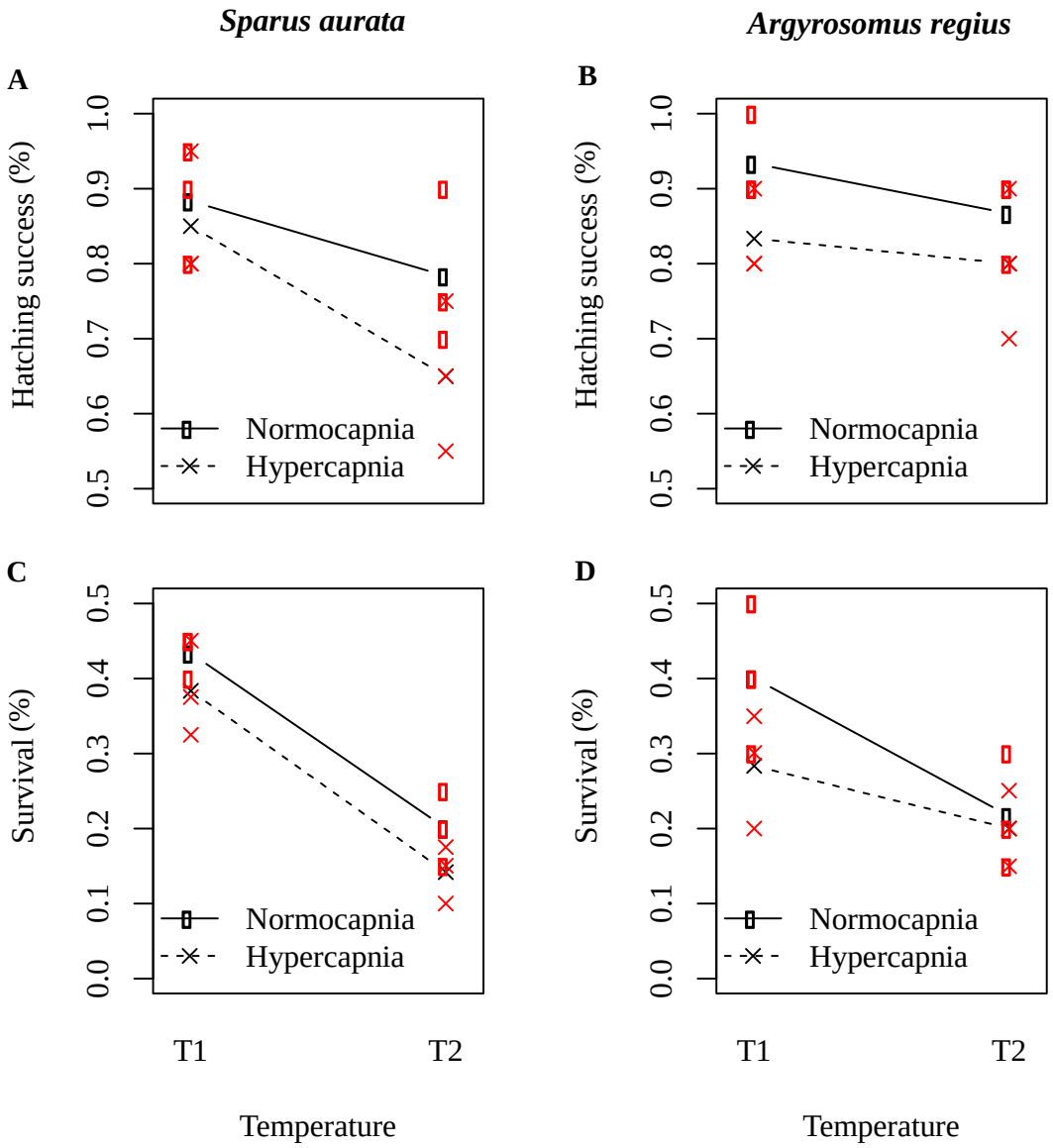


Figure 2

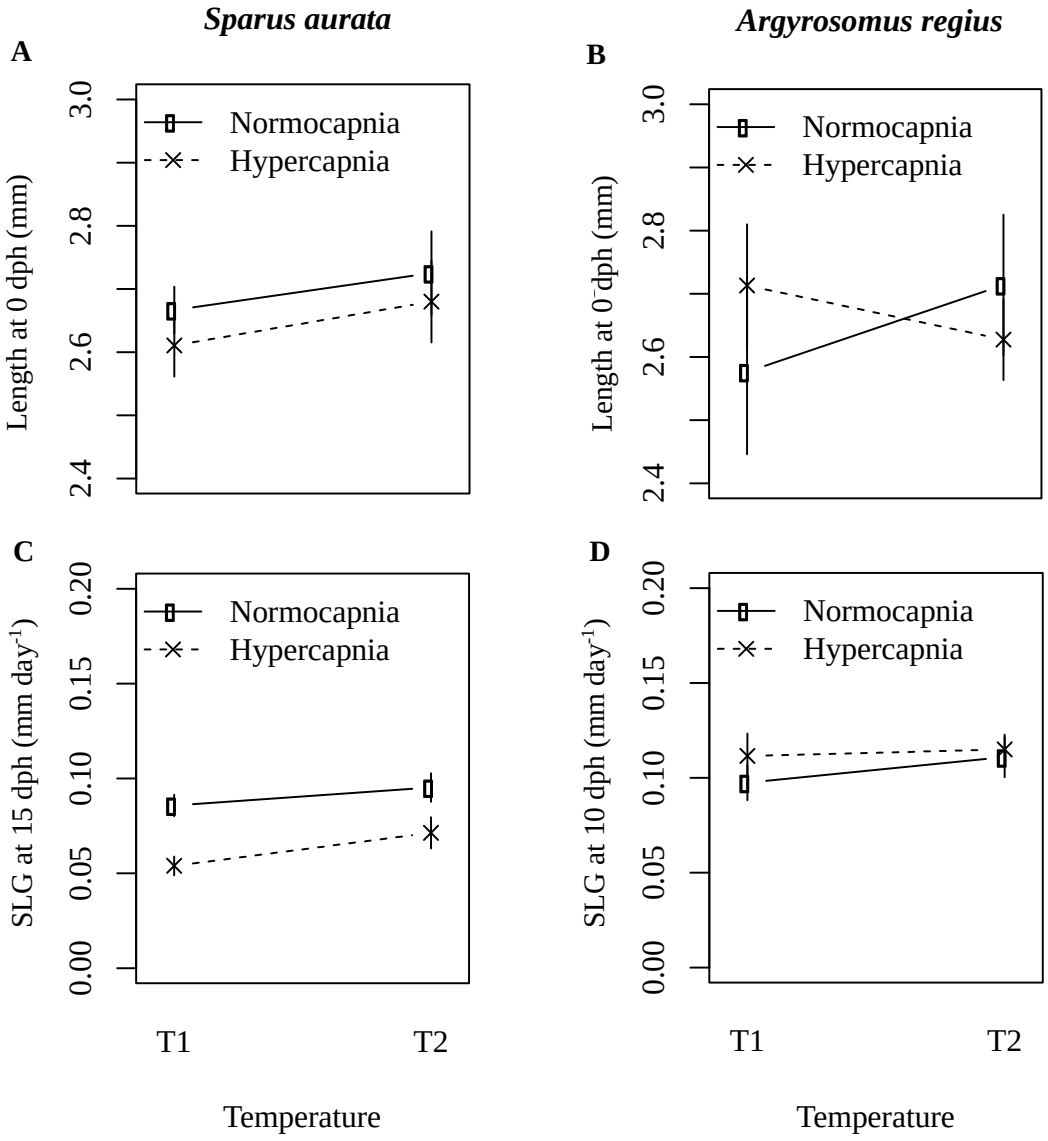

Sparus aurata
Argyrosomus regius
A
B
C
D
Normocapnia
Hypercapnia
Length at 0 dph (mm)
SLG at 15 dph (mm day-1)
SLG at 10 dph (mm day-1)
2.4
2.6
2.8
3.0
0.00
0.05
0.10
0.15
0.20
T1
T2
Temperature

Figure 3

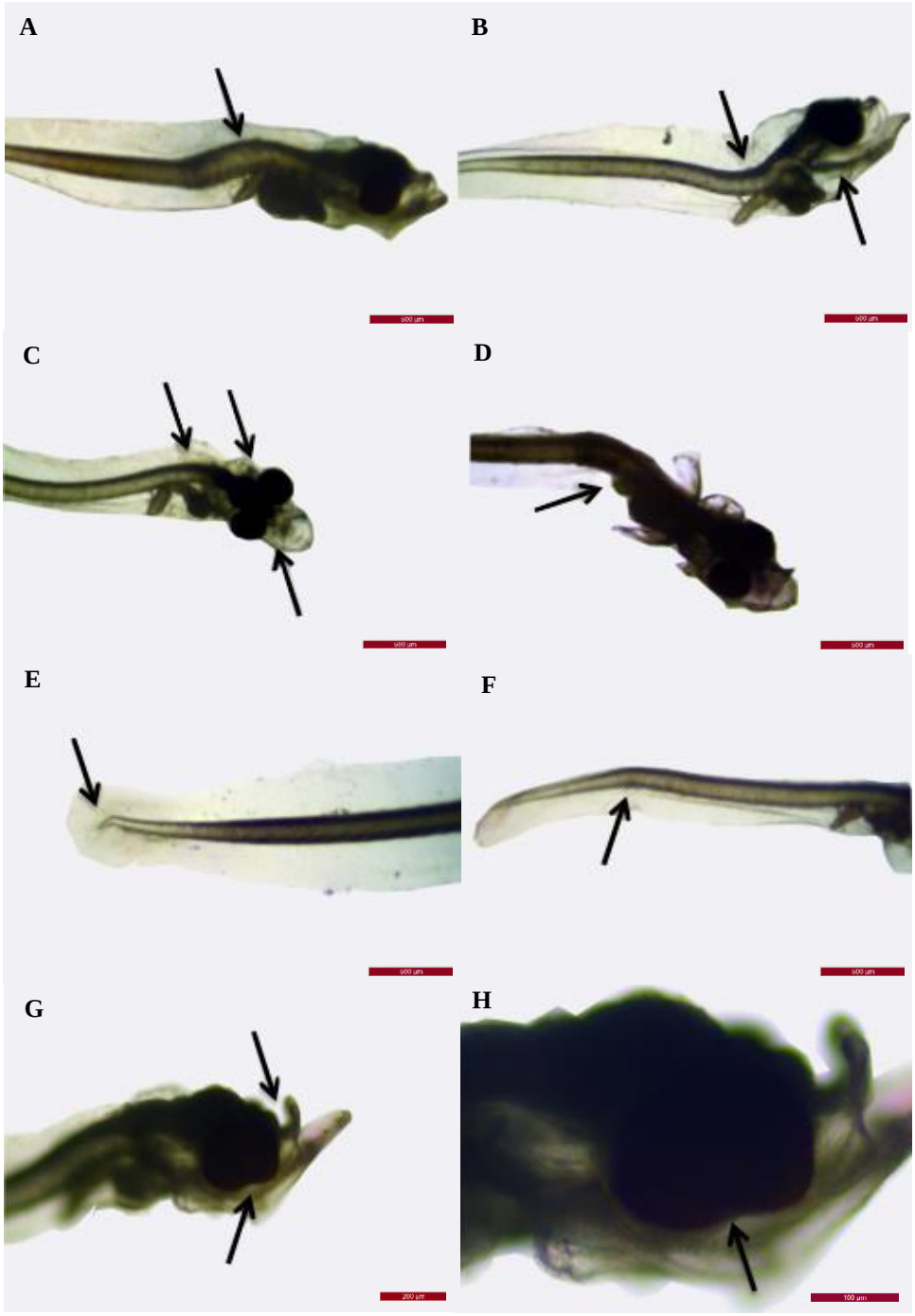


Figure 4

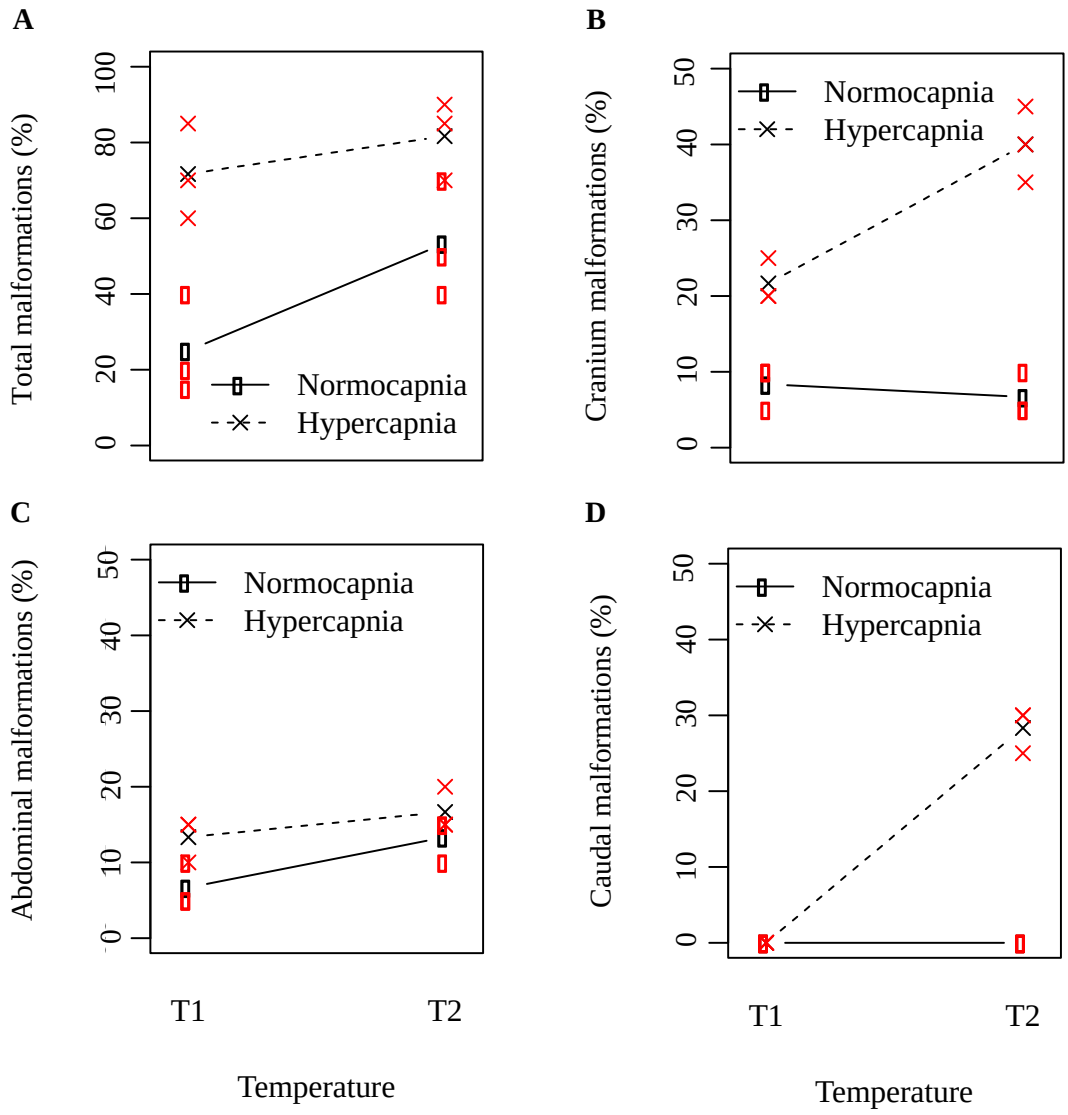
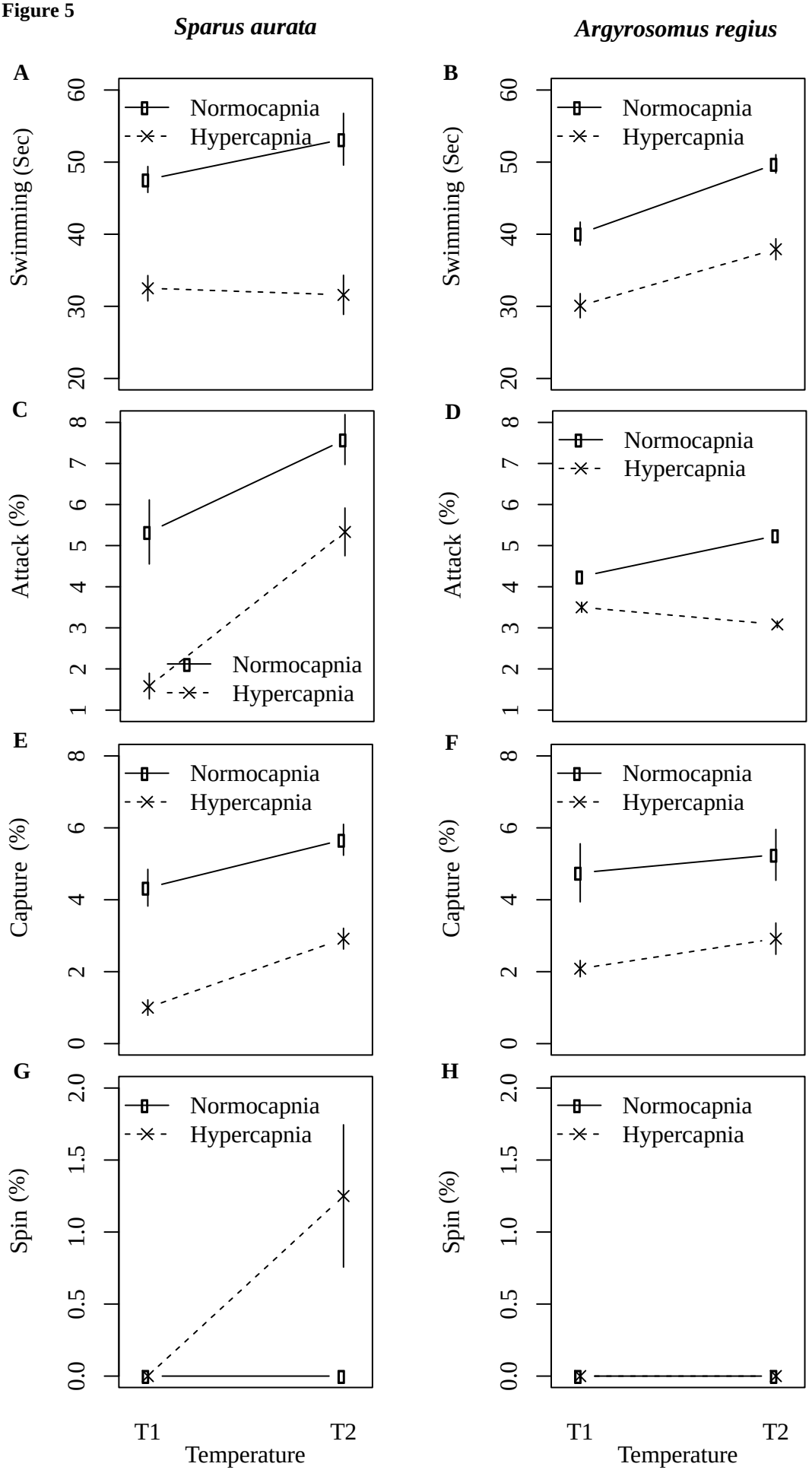

A
Total malformations (%)
0
20
40
60
80
100
Normocapnia
Hypercapnia
B
Cranium malformations (%)
0
10
20
30
40
50
Normocapnia
Hypercapnia
C
Abdominal malformations (%)
0
10
20
30
40
50
Normocapnia
Hypercapnia
T1
T2
Temperature
D
Caudal malformations (%)
0
10
20
30
40
50
Normocapnia
Hypercapnia
T1
T2
Temperature

Figure 5


Sparus aurata
Argyrosomus regius
A
B
C
D
E
F
G
H
Swimming (Sec)
Attack (%)
Capture (%)
Spin (%)
Normocapnia
Hypercapnia
T1
T2
Temperature