



**HAL**  
open science

## Food deprivation reduces social interest in the European sea bass *Dicentrarchus labrax*

Cassandre Aimon, Nicolas Le Bayon, Stephane Le Floch, Guy Claireaux

### ► To cite this version:

Cassandre Aimon, Nicolas Le Bayon, Stephane Le Floch, Guy Claireaux. Food deprivation reduces social interest in the European sea bass *Dicentrarchus labrax*. *Journal of Experimental Biology*, 2019, 222 (3), pp.UNSP jeb190553. 10.1242/jeb.190553 . hal-02749009

**HAL Id: hal-02749009**

**<https://hal.science/hal-02749009>**

Submitted on 3 Jun 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## RESEARCH ARTICLE

# Food deprivation reduces social interest in the European sea bass *Dicentrarchus labrax*

Cassandre Aimon<sup>1,2,\*</sup>, Nicolas Le Bayon<sup>3</sup>, Stéphane Le Floch<sup>2</sup> and Guy Claireaux<sup>1</sup>

## ABSTRACT

Periods of food deprivation of several months are common events for fishes and in such conditions, fitness will be determined by their capacity to maximize food encounters while minimizing predation risk. In this context, the propensity to take risks and the willingness to associate with conspecifics are particularly important as they contribute to alleviating the trade-off between predation avoidance and foraging efficiency. This study examined to what extent food deprivation modulates fish risk-taking and social behaviours, as well as the relationship between them. To address these issues, juvenile European sea bass were either fed daily with a maintenance ration or food deprived for a period of 3 weeks. Risk taking and sociability were assessed through measurements of fish willingness to explore a novel environment, and to interact with a novel object or a conspecific. Multivariate analysis allowed the identification of three behaviours: risk taking, exploratory activity and solitariness. Food-deprived fish interacted less with conspecifics than control fish; however, no difference in terms of risk taking and exploratory patterns was observed. Finally, the relationship between risk taking and solitariness was influenced by feeding status. When food-deprived, fish with a higher propensity to take risk displayed increased solitariness, while when fed normally, they interacted more with conspecifics.

**KEY WORDS:** Behaviour, Teleost fish, Exploratory activity, Sociability, Risk taking, Behavioural interaction

## INTRODUCTION

Food availability and accessibility are key determinants of fitness (Patrick et al., 2017). In aquatic ecosystems, the finding and capture of prey are not guaranteed and periods of starvation of several months are not unusual (Gingerich et al., 2010). In such conditions, animals preserve their fitness through behavioural responses that maximize food encounter and capture rates while minimizing predation risk (Lima and Dill, 1990). When food is scarce, increasing the duration and extent of food searching, further away from a shelter, for instance, is a risk-prone behaviour which favours food encounter. The drawback of such behaviour is increased predator encounter rate and, therefore, increased mortality risk (Biro and Stamps, 2010; Lima and Dill, 1990). In contrast, risk-averse behaviours, such as staying in a protective area, benefit the individual through energy saving and reduced predation risk. The

downside of this strategy is obviously a reduction in feeding opportunities (Krause and Ruxton, 2002).

Risk taking has major consequences for an individual's fitness as it has been shown to correlate with growth, energy metabolism, dispersal, breeding success, offspring nourishment and social dominance (Ariyomo and Watt, 2012; Bell and Sih, 2007; Brown et al., 2005; Cote et al., 2010; Dingemanse et al., 2004; Greenberg and Mettke-Hofmann, 2001; Huntingford et al., 2010; Jolles et al., 2015; Mutzel et al., 2013; Rudin and Briffa, 2012). Risk-prone individuals accept higher risk in return for gaining information and possibly increased reward. Risk-averse individuals, in contrast, tend to avoid potentially risky situations, accepting reduced gains in return for lower risk. However, the willingness of an individual to take risk is context dependent (Coleman and Wilson, 1998). For instance, Galhardo et al. (2012) showed that social context influences risk taking of male cichlid fish (*Oreochromis mossambicus*), with fish in the presence of familiar conspecifics being more prone to accept risk than fish in the presence of unfamiliar conspecifics or in social isolation. This context dependency of risk taking is the cause of disagreement among authors regarding the most appropriate methodology to assess it (Carter et al., 2013; Conrad et al., 2011; Réale et al., 2007; Wilson et al., 1993). While some authors restrict the measures of risk acceptance to the context of predation (Réale et al., 2007), others extend the relevance of the notion to any context where an animal has to make a decision towards unfamiliar and potentially dangerous stimuli (Frost et al., 2013; Leblond and Reeb, 2006; Nakayama et al., 2012; Toms et al., 2010; Wilson et al., 1993). In the present study, risk taking was considered in its broader sense, with exploratory tendency and neophilia been recognized as components of an individual's willingness to take risk.

Sociability is an important modulator of fish behavioural responses to the environmental context and, particularly, of the trade-off between maximizing foraging opportunities and minimizing predator encounter. Sociability is classically defined as one animal's reaction to conspecifics, excluding aggressive interactions (Conrad et al., 2011). Fish express sociability in a species-specific manner, one example of which is the willingness to shoal. Shoaling behaviour is considered as a flexible strategy by which individuals increase food detection while lowering predation risk through additive vigilance, numerical risk dilution and predator confusion effect (Clark and Mangel, 1986; Godin, 1986; Krause and Ruxton, 2002; Krause et al., 2000a; Pitcher and Parrish, 1993; Pulliam and Caraco, 1984). However, shoaling is also associated with increased competition within the group (Pitcher, 1986; Pulliam and Caraco, 1984). Shoal formation relies on the decision of individuals to remain together and it has been proposed that food restriction influences individual decisions regarding self-assembly with conspecifics (Frommen et al., 2007; Raubenheimer et al., 2012). For instance, Krause (1993), Arber et al. (1995) and Hensor et al. (2003) reported that food-deprived fish tended to spend more time alone than well-fed individuals. However, at least one study

<sup>1</sup>Université de Bretagne Occidentale, LEMAR (UMR 6539), Unité PFOM-ARN, Centre Ifremer de Bretagne, 29280 Plouzané, France. <sup>2</sup>CEDRE, Research Department, 715 rue Alain Colas, CS 41836, Brest 29218-Cedex 2, France.

<sup>3</sup>Ifremer, LEMAR (UMR 6539), Unité PFOM-ARN, Centre Ifremer de Bretagne, 29280 Plouzané, France.

\*Author for correspondence (cassandre.aimon@gmail.com)

 C.A., 0000-0003-3800-945X

described the reverse response (Killen et al., 2016). Sociability characterizes an animal's inclination for social interaction such as shoaling. In the present study, solitariness was used as the reciprocal of sociability, with high solitariness reflecting an individual's unwillingness to group with conspecifics and low solitariness indicating an individual's inclination for social interaction.

Analysing behaviours is a key component in understanding how fish populations respond to seasonal and stochastic variation in environmental conditions. Equally important is the interaction between those behaviours. The relationship between risk taking and sociability has been examined in fish, and whereas a relationship was found in some species (*Symphodus ocellatus*: Budaev, 1997; *Gasterosteus aculeatus*: Ward et al., 2004), it was not observed in others (*Danio rerio*: Moretz et al., 2007). Moreover, when found, this relationship displayed different forms depending on the species considered. In most species examined, a negative correlation between risk taking and sociability has been reported (*S. ocellatus*: Budaev, 1997; *G. aculeatus*: Ward et al., 2004; Harcourt et al., 2009a,b; *Lepomis gibbosus*: Wilson et al., 1993). In the mosquitofish (*Gambusia affinis*: Cote et al., 2010) as well as in two terrestrial ectotherms, red ants (*Myrmica* spp.: Chapman et al., 2011) and the European green lizard (*Lacerta vivipara*: Cote and Clobert, 2007), the reverse was observed, with a positive correlation between risk taking and sociability. It has been proposed that correlations among behaviours might be adaptive as they contribute to individual fitness (Bell, 2005; Bell and Sih, 2007; Bell and Stamps, 2004). In the three-spined stickleback, for example, no interaction between risk taking (swimming activity after a predator attack) and aggressiveness (towards conspecifics) was observed under low predation pressure. Under high predation pressure, however, a positive correlation between these behaviours was observed (Bell, 2005). Although disappointing, these conflicting results may not be all that surprising as there is no reason why relationships between behaviours should all have the same form across vastly different experimental systems using species with different ecologies and behavioural repertoires. Thus, to better assess the true adaptive value of fish behavioural strategies, future studies should examine the relationships between behavioural traits, with particular consideration for the strength and form of these relationships.

Using juvenile European sea bass, *Dicentrarchus labrax*, as a model species, the present study investigated to what extent food deprivation modulates fish risk-taking and social behaviours, as well as the relationship between them. At the juvenile stage, European sea bass form shoals that occupy shallow coastal habitats where spatial and seasonal fluctuations in prey availability are common, exposing them to periods of starvation (Claireaux et al., 2013; Dupont-Prinet et al., 2010). Four commonly used behavioural tests were implemented, with the initial anticipation that they would allow assessment of risk taking and sociability through measuring the willingness of individuals to explore a novel environment, to interact with a novel object or a conspecific and to use a shelter. Behavioural responses of 3 week food-deprived fish were compared with those of control individuals fed daily with a maintenance ration. Three hypotheses were tested: (1) starvation favours solitariness to avoid competition for food, (2) starvation increases risk taking and exploratory activity to enhance food encounters and (3) food deprivation modulates the relationships between solitariness, exploratory activity and risk taking.

## MATERIALS AND METHODS

### Study animals

Fifty-six juvenile European sea bass, *Dicentrarchus labrax* (Linnaeus 1758) (age 0<sup>+</sup>, mass 14.6±0.08 g, mean±s.e.m.), were obtained from a local fish farm (Aquastream, Lorient, France).

Upon arrival at the laboratory (Ifremer, Brest, France), fish were anaesthetized (MS-222; 20 mg l<sup>-1</sup>), measured for total length and mass, and implanted subcutaneously with an identification tag (RFID; Biolog-id, Bernay, France). Fish were then placed in a 2000 l indoor tank supplied with open-flow, thermoregulated (20°C) and fully aerated seawater (salinity 32 ppt). Artificial lighting followed local photoperiod. Fish were fed daily with a maintenance ration (1% body mass) using commercial feed (Neo Start Coul 2, Le Gouessant, Lamballe, France). The experiments were approved by the French Ethics Committee in charge of Animal Experimentation no. 74 (permit number: APAFIS#3814-2016012715396101 v2) and were in accordance with institutional guidelines. The experimental procedures were non-invasive.

### Experimental protocol

Fish were acclimated to the laboratory conditions for 2 months before the following procedure was implemented. On alternate weeks a set of eight fish was randomly selected from the holding tank, anaesthetized (MS-222; 20 mg l<sup>-1</sup>), measured for total length and mass and transferred to one of two treatment tanks (50 l). These treatment tanks were situated in the experimental room and received the same water and light conditions as the original holding tank. The two experimental treatments were a control treatment, in which fish were fed normally, and a food-deprived (FD) treatment, in which fish were not fed during a 3 week period.

Following the 3 weeks of either control or FD treatment, a set of eight fish from one experimental treatment tank was subjected to the following protocol (Fig. S1). Fish were gently placed (without emersion) into one of eight individual confinement chambers. These chambers consisted of an opaque PVC tube (13 cm×5 cm length×diameter) closed at both ends with plastic meshing to allow water renewal inside the chamber. These chambers were then placed side by side on the bottom of a recovery tank and fish were left undisturbed for an additional 24 h. The eight fish were then successively subjected to a sequence of four consecutive trials over a period of 4 days. On each testing day, the running order of the eight fish was randomized. Each chamber was smoothly moved from the recovery tank to the experimental arena using a 2 l plastic container filled with water. After 3 min to allow the fish to recover from potential disturbance arising from the transfer, one end of the tube was opened from a distance and the fish were allowed 1 min to exit the chamber. In most cases (49/56), the tube had to be gently lifted to encourage the fish to swim out. Following their entrance in the arena, nearly all fish displayed a period of agitation which typically lasted less than a minute. To avoid including this 'flight response' in our analysis, the first minute following fish entry in the testing arena was not taken into consideration during video analysis.

The testing arena consisted of a white rectangular shallow tank (156 cm×99 cm×14 cm, length×width×height, respectively). A curtain placed around and over the arena screened fish from visual disturbance. The arena was filled with the same water as the rearing and treatment tanks and was homogeneously lit (30 lx) using neon lamps.

A camera (Logitech webcam C930e) situated 1 m above the water surface allowed the recording (15 frames s<sup>-1</sup>) of fish movements during the 30 min that followed fish entry into the arena. Following the 30 min trial, the tested fish was returned to its PVC tube, which was then placed back into a new recovery tank until the next day. This recovery tank allowed separation of the already tested fish from those waiting to be tested, preventing the transfer of chemical cues between fishes. Water conditions in the recovery tanks were identical to those in the treatment tank. Once a fish had been removed, the arena was emptied, refilled and the next fish brought in.

Experimental sets of fish were successively subjected to the following sequence. On day 1, fish were subjected to a novel environment test which consisted of exposing fish to a bare tank. On day 2, a novel object was placed at the centre of the arena prior to the introduction of the fish. The object consisted of a white, opaque polypropylene jar (13 cm height, 10.5 cm in diameter) with holes (1.5 mm) all around. The object was placed in such a way that no shadow was created in the arena. On day 3, a conspecific was placed in the opaque jar and 20 min later the tested fish was transferred in the arena. The perforations in the jar allowed the transfer of chemical cues and pressure waves related to movement but its opacity prevented visual contact with the focal fish and the establishment of dominant–subordinate relationships. Finally, on day 4, a refuge, which consisted of a flat piece of black plastic (19 cm×19 cm, length×width), was placed on the bottom in one corner of the arena, opposite the fish release site (Nelson and Claireaux, 2005). This fixed temporal order of the four assays allowed the potential carry-over effects between trials carried out on successive days to be kept the same. In addition, this order was established to familiarize the fish to one condition before testing the following one, introducing only one change in each test. The day 1 assay allowed the fish to familiarize itself with the arena, which was then no longer considered as a novelty on day 2. On day 2, the novel object was added as the only source of novelty in the familiar arena. On day 3, the fish was already familiar with this object, allowing evaluation of the fish's interest in a conspecific through the addition of a non-focal fish inside the jar. This individual was used as a social stimulus for the whole day (8 trials). It was taken from the holding tank and was placed inside the polypropylene jar 20 min prior to the beginning of the experiment. Finally, on day 4, refuging behaviour was tested by evaluating the fish's capacity to localize and use a refuge in an already well-explored arena. At the end of the week, the eight fish were anaesthetized (MS-222; 20 mg l<sup>-1</sup>) and their total length and body mass measured to estimate specific growth rate. They were then returned to their original holding tank.

### Behavioural analyses

On day 1, four indices of activity were measured during the first 5 min of the test (i.e. the total time spent swimming,  $T_{\text{swim}}$ ; the number of sections crossed,  $N_{\text{SC}}$ ; the time spent in the central section of the arena,  $T_{\text{central}}$ ; and the number of entries into the central section,  $N_{\text{ent,cent}}$ ). To score  $N_{\text{SC}}$ , a grid was placed over the screen of the computer used for video analysis; this grid divided the arena in 16 sections of equal dimension (39 cm×24.75 cm). To score  $T_{\text{central}}$ , the central section (78 cm×49.5 cm) was distinguished from the periphery of the arena. Analysis on day 2 consisted of measuring, during the first 5 min period of the test, four new indices of activity (i.e. the latency to the first approach to the novel object,  $L_{\text{app,NO}}$ ; the time spent close to the novel object,  $T_{\text{NO}}$ ; the number of approaches to the novel object,  $N_{\text{app,NO}}$ ; and the number of revolutions around the novel object,  $N_{\text{rev,NO}}$ ). In this test, the fish was considered to be close to the conspecific when it was less than a body length away from it. During day 3, the variables of interaction with the conspecific were measured during the last 5 min of the 30 min test to ensure that the focal fish detected the conspecific. During this test, the same four variables as during day 2 were measured with respect to the conspecific (i.e. the latency to approach the conspecific,  $L_{\text{app,con}}$ ; the time spent close to it,  $T_{\text{con}}$ ; the number of approaches to the conspecific,  $N_{\text{app,con}}$ ; and the number of revolutions around the conspecific,  $N_{\text{rev,con}}$ ). Again, the fish was considered to be close to the object containing the conspecific when it was less than a body length away from it. Finally, analysis on day

4 consisted of recording the following three indices over the 30 min of the test (the latency to enter the shelter,  $L_{\text{shelter}}$ ; the time spent in the shelter,  $T_{\text{shelter}}$ ; and the number of exits from the shelter,  $N_{\text{exit,shelter}}$ ). Note that the same observer scored the different behaviours by visual observation from the videos.

### Statistical analysis

Two fish were excluded from the analyses because video recordings failed (total of 54 fish).

Owing to our experimental design, one possible option was to analyse each test separately, as in Dingemans et al. (2010), for example. We opted for a more integrative approach using a principal components analysis (PCA) that regrouped, on the same axes, the variables that assessed a common behaviour. Three principal components (PCs) were chosen according to Kaiser's criterion (Kaiser, 1961), retaining only factors with eigenvalues greater than 1. Linear combination of the variables was used to label the three PCs: risk taking (PC1), exploratory activity (PC2) and solitariness (PC3). Behavioural scores of FD and control fish were compared using parametric multivariate analysis of variance (MANOVA), followed by ANOVA tests to generate univariate statistics. A linear model was fitted to compare the relationship between risk taking and sociability in control and FD fish. In this model, feeding treatment, risk taking (PC1) and exploratory activity (PC2) including the interactions treatment×(PC1+PC2), were used as explanatory factors influencing the sociability (PC3), which was used as the response variable. A backward stepwise reduction of the full model was then applied to exclude non-significant interactions.

All statistical analyses were performed using R version 3.3.1 (<http://www.R-project.org/>) with the package FactoMineR (function PCA), with all variables scaled, and the package stats (functions manova, summary.aov and lm). Model diagnostics were evaluated using graphical procedure (Q–Q plot). Statistical significance was accepted at  $P<0.05$ .

### RESULTS

Data were collected from  $N=30$  control and  $N=23$  FD fish. Mean initial mass was 14.6±0.10 g and 14.5±0.16 g for the control and FD fish, respectively. Mean specific growth rate measured over the 3 week treatment period was 0.9±0.07% day<sup>-1</sup> and -0.3±0.03% day<sup>-1</sup> for the control and FD fish, respectively.

### Reduction and structure of the variables

The impact of starvation on the behaviour of juvenile sea bass was analysed using two successive PCA. The first PCA included the 15 variables extracted from the novel environment (day 1), novel object (day 2), conspecific (day 3) and shelter (day 4) experimental trials. The variables that made a lower contribution to the construction of the PCA axes than the mean contribution of the 15 variables initially tested were then removed and a second PCA was conducted (Cibois, 1986, 1997). In this process, the three variables measured during the shelter trial (day 4) were excluded. Moreover, one individual with a contribution to the structure of the axes that was 20 times higher than the median contribution of all the individuals was considered as an outlier and removed from the analysis. In this second PCA, three PCs were selected (eigenvalue>1) which represented 75.16% of the total variance in our dataset (Table 1). The three principal components were respectively interpreted as indicators of risk taking, exploratory activity and solitariness. This labelling resulted from the specific linear combination of variables on each axis. PCs are described in the following paragraphs.

**Table 1. Eigenvalues of the axes of the principal components analysis (PCA)**

| PC | Eigenvalue | % Variance | Cumulative % variance |
|----|------------|------------|-----------------------|
| 1  | 4.157      | 34.639     | 34.639                |
| 2  | 3.049      | 25.404     | 60.043                |
| 3  | 1.814      | 15.114     | 75.157                |
| 4  | 0.912      | 7.604      | 82.761                |
| 5  | 0.625      | 5.211      | 87.972                |

The PCA was performed with the 12 behavioural variables extracted from the novel environment (day 1), novel object (day 2) and conspecific (day 3) trials.

PC1 explained 34.64% of the variability. This axis was labelled ‘risk taking’ as it incorporated eight variables measuring a fish’s propensity to take risk when exposed to novelty. These included the three variables measuring the interaction with the novel object (day 2:  $T_{NO}$ ,  $N_{app,NO}$ ,  $N_{rev,NO}$ ) and the three variables measuring the interaction with the newly introduced conspecific within the familiar object (day 3:  $T_{con}$ ,  $N_{app,con}$ ,  $N_{rev,con}$ ). Also included here are the two variables measuring the latency before the first approach to the novel object/conspecific ( $L_{app,NO}$  and  $L_{app,con}$ , respectively). The first six variables correlated positively with PC1 while the last two correlated negatively with PC1 (Table 2). For PC1, positive scores indicated risk-prone individuals, while negative scores indicated risk-averse individuals.

PC2 explained 25.40% of the total variance. This axis was labelled ‘exploratory activity’ as it was defined by four variables measuring fish activity during exploration of the novel environment on day 1 (i.e.  $T_{swim}$ ,  $N_{SC}$ ,  $T_{central}$ ,  $N_{ent,cent}$ ). These four variables correlated positively for this axis (Table 2). For PC2, individuals with a higher exploratory tendency had positive scores, while those with a lower tendency to explore had negative scores.

PC3 explained 15.11% of the variability. This axis was labelled ‘solitariness’ as it was defined by two sets of variables that distinguished interaction with the empty object (day 2) from interaction with the object in the presence of a conspecific within that object (day 3). The first set of variables included  $T_{NO}$ ,  $N_{app,NO}$  and  $N_{rev,NO}$  and it correlated positively with PC3. The second set

**Table 2. Coefficients of correlation of the three first principal components (PCs) for each variable**

| Variable       | PC1 risk taking | PC2 exploratory activity | PC3 solitariness |
|----------------|-----------------|--------------------------|------------------|
| $N_{SC}$       | 0.108           | <b>0.878</b>             | 0.207            |
| $T_{swim}$     | -0.044          | <b>0.744</b>             | 0.351            |
| $T_{central}$  | 0.012           | <b>0.748</b>             | 0.171            |
| $N_{ent,cent}$ | 0.035           | <b>0.894</b>             | 0.178            |
| $L_{app,NO}$   | <b>-0.744</b>   | 0.075                    | -0.147           |
| $N_{app,NO}$   | <b>0.748</b>    | -0.223                   | <b>0.521</b>     |
| $T_{NO}$       | <b>0.689</b>    | -0.257                   | <b>0.575</b>     |
| $N_{rev,NO}$   | <b>0.682</b>    | -0.302                   | <b>0.448</b>     |
| $L_{app,con}$  | <b>-0.761</b>   | -0.146                   | 0.290            |
| $N_{app,con}$  | <b>0.693</b>    | 0.297                    | <b>-0.476</b>    |
| $T_{con}$      | <b>0.759</b>    | 0.066                    | <b>-0.392</b>    |
| $N_{rev,con}$  | <b>0.674</b>    | 0.202                    | <b>-0.546</b>    |

$N_{SC}$ , number of sections crossed;  $T_{swim}$ , total time spent swimming;  $T_{central}$ , time spent in the central section of the arena;  $N_{ent,cent}$ , number of entries into the central section of the arena;  $L_{app,NO}$ , latency to the first approach to the novel object;  $N_{app,NO}$ , number of approaches to the novel object;  $T_{NO}$ , time spent close to the novel object;  $N_{rev,NO}$ , number of revolutions around the novel object;  $L_{app,con}$ , latency to approach the conspecific;  $N_{app,con}$ , number of approaches to the conspecific;  $T_{con}$ , time spent close to the conspecific;  $N_{rev,con}$ , number of revolutions around the conspecific. These three PCs represent 75.16% of the variance in the data. The variables used for the delineation of each component are in bold.

included  $T_{con}$ ,  $N_{app,con}$  and  $N_{rev,con}$  and it correlated negatively with PC3 (Table 2). For PC3, solitary individuals had positive scores, while individuals with more social interest displayed negative scores. It is important to point out, however, that these variables also loaded heavily on PC1. PC3 should therefore be interpreted with caution. Nevertheless, it allowed us to explore fish social behaviour by differentiating interaction with the empty object from interaction with a conspecific inside this object.

To summarize, when transferred to the experimental arena, fish that scored highly on PC1 took less time to make the first approach to the object in the centre (day 2 and day 3; low  $L_{app,NO}$  and  $L_{app,con}$ ) and spent more time close to it (day 2 and day 3; elevated  $T_{NO}$ ,  $N_{app,NO}$ ,  $N_{rev,NO}$ ,  $T_{con}$ ,  $N_{app,con}$  and  $N_{rev,con}$ ) (Fig. 1A–D). In contrast, fish that scored highly on PC2 spent less time motionless (elevated  $T_{swim}$ ), moved greater distances (elevated  $N_{SC}$ ) and explored the central zone of the arena more actively (elevated  $T_{central}$  and  $N_{ent,cent}$ ) (Fig. 1A,B,E,F). Finally, fish that scored highly on PC3 were more in contact with the object when it represented a novelty (day 2; elevated  $T_{NO}$ ,  $N_{app,NO}$  and  $N_{rev,NO}$ ) but had minimal interaction with the central object when it contained a conspecific (day 3; low  $T_{con}$ ,  $N_{app,con}$  and  $N_{rev,con}$ ) (Fig. 1C–F).

### Effects of the feeding regime

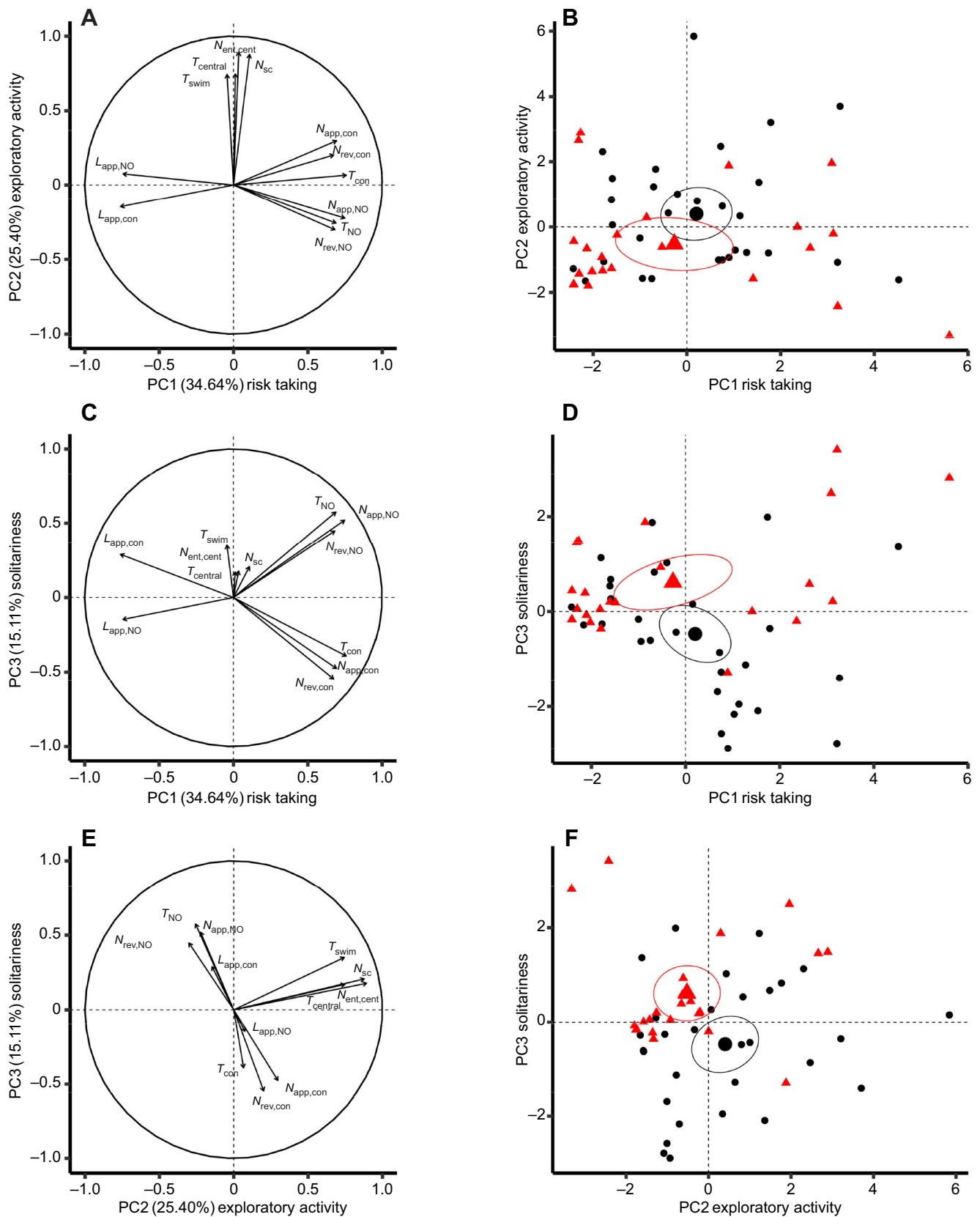
MANOVA showed that, overall, the effect of the feeding regimes on fish behaviour was significant (Pillai’s trace=0.242,  $F_{1,49}=5.202$ ,  $P=0.003$ ). Specifically, fish from the two feeding regimes showed no statistically different scores on PC1 (Fig. 1B,D; risk taking,  $F_{1,51}=0.678$ ,  $P=0.414$ ). However, they showed nearly statistically different scores on PC2, the significance threshold being almost attained (Fig. 1B,F; exploratory activity,  $F_{1,51}=3.784$ ,  $P=0.057$ ). Finally, the two feeding treatments yielded statistically different scores on PC3 (Fig. 1D,F; solitariness,  $F_{1,51}=9.670$ ,  $P=0.003$ ), with FD fish displaying higher scores than control fish, meaning that FD fish interacted less with the conspecific than did well-fed fish.

### Factors influencing solitariness

Individual degree of risk taking was expected to influence social behaviour. This relationship was investigated using a linear modelling approach (Table 3). The resulting model indicated that PC3 (solitariness) was influenced by PC1 (risk taking) differently according to the feeding treatment ( $F_{4,48}=5.423$ ; treatment $\times$ PC1 risk taking interaction,  $P=0.004$ ; Table 3). Fig. 2 illustrates the different correlations between scores on PC1 and PC3 according to the feeding treatment. These scores correlated positively for FD fish ( $P=0.022$ ), while they displayed a nearly significant negative correlation in control fish ( $P=0.064$ ). Despite these differences between feeding treatment groups, it should be noted that at the left end of PC1 (risk-averse individuals), fish were indistinguishable from one another with regard to their social interest (PC3). However, the solitariness level of individuals progressively differentiated according to their feeding regime as their scores on PC1 increased (Fig. 2).

### DISCUSSION

The aim of the present study was to examine to what extent sea bass nutritional status modulates risk taking and sociability. Three hypotheses were tested: (1) starvation favours solitariness to reduce competition for food, (2) starvation increases exploratory activity and risk taking to enhance food encounters and (3) food deprivation modulates the relationships between solitariness, exploratory activity and risk taking. In accordance with our first hypothesis, food deprivation modulated the interaction with a conspecific, with FD fish interacting less with conspecifics than did control fish.



**Fig. 1. Position of individuals along the risk-taking, exploratory activity and solitariness axes of the PCA. (A,C,E)** Relationships between behavioural variables and their contribution to the principal component (PC). (B,D,F) Factor maps for individual fish. Top row: exploratory activity versus risk taking; middle row: solitariness versus risk taking; bottom row: solitariness versus exploratory activity. Red triangles: food-deprived fish ( $N=23$ ); black circles: control fish ( $N=30$ ). Confidence ellipses are plotted around group mean points.

**Table 3. Linear model describing the interaction between feeding regime, risk taking and solitariness**

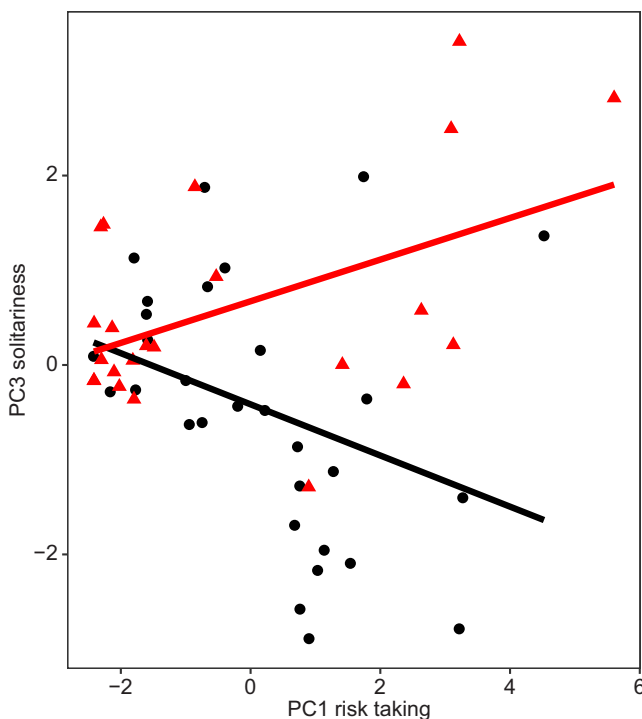
| Included variables        | Excluded variables                 | Estimate | s.e.  | t-value | P-value |
|---------------------------|------------------------------------|----------|-------|---------|---------|
| (Intercept)               |                                    | -0.416   | 0.217 | -1.915  | 0.061   |
| Treatment                 |                                    | 1.088    | 0.329 | 3.305   | 0.002** |
| PC1 risk taking           |                                    | -0.270   | 0.129 | -2.097  | 0.041*  |
| Treatment×PC1 risk taking |                                    | 0.490    | 0.164 | 2.981   | 0.004** |
|                           | PC2 exploratory activity           | 0.117    | 0.096 | 1.212   | 0.232   |
|                           | Treatment×PC2 exploratory activity | -0.090   | 0.202 | -0.446  | 0.657   |

Estimates, s.e. (standard error), *t*-value and *P*-value were generated by the linear models fitting procedure. Included variables refer to those that made a significant contribution to the model; excluded variables are those that made a non-significant contribution to the model. Significant effect of the variables on PC3 (solitariness): \**P*<0.05, \*\**P*<0.01.

However, contrary to our second hypothesis, control and FD fish did not differ in terms of risk taking and exploratory patterns. In agreement with our third hypothesis, we observed that feeding status influenced the relationship between risk taking and solitariness, with risk-prone fish displaying increased solitariness when food restricted and reduced solitariness when fed normally.

### Behaviours

Using PCA, the impact of feeding status on the fish's response to novelty was explored. Through this approach, the 15 measured variables were combined into smaller sets of interpretable linear combinations (components). Three components were identified that explained approximately 75% of the variability of fish behavioural patterns. The first component was considered to indicate risk taking as it included variables usually attributed to the propensity to take risk when faced with novelty. The second component of our PCA was termed exploratory activity as it informed about fish swimming activity level and pattern while exploring a novel environment. The third component was considered to specify fish level of solitariness as it was defined by measures of an individual's unwillingness to group with a conspecific.



**Fig. 2. Relationships between PC1 risk taking and PC3 solitariness according to the feeding treatment.** Red triangles and lines: food-deprived fish (*N*=23); black circles and lines: control fish (*N*=30).

Because of terminological and methodological inconsistencies, the delineation of behaviours can be somewhat subjective, potentially resulting in conflicting interpretations (Carter et al., 2013; Roche et al., 2016; Toms et al., 2010). The current dataset provides a new illustration of the difficulty of contextually interpreting and labelling behavioural responses. The exploration of the central zone in a novel environment test as well as the response to a novel object during the novel object test are classically used to assess an individual's degree of risk taking in response to novelty (Boulton et al., 2014; Budaev, 1997; Burns, 2008; Frost et al., 2013; Sneddon, 2003; Wilson et al., 1993; Wright et al., 2003). In the present study, however, variables from these two tests did not correlate with each other, indicating that they may not evaluate the same behaviour. Whereas variables from the novel object test correlated on PC1, the number of entries into the central zone and the duration of stays in this area correlated with the total time spent swimming and the number of sections crossed in the novel environment test on PC2. This linear combination on PC2 supports the hypothesis that the number of entries and the time spent in the central zone may simply be proportional to an individual's activity level, with more-active fish passing more often and spending more time in the centre of the experimental arena than less-active fish. We suggest that those four variables are indicators of exploratory activity level rather than indicators of risk taking.

One major requirement to define an animal's personality trait is that the inter-individual differences in the corresponding behavioural measure must be consistent. However, demonstrating consistency requires that the measure is repeated several times in exactly the same context. In the present experiment, this was clearly not the case, as fish behaviours were measured only once. Consistent with the fact that the repeatability of fish responses was not evaluated, we considered that the tests used in the present study evaluated the impact of starvation on behavioural tendencies (risk taking, exploratory activity and solitariness) and not on personality traits (boldness–shyness, exploration–avoidance, sociable–solitary).

### Effect of food deprivation

The effect of a 3 week food deprivation period on solitariness, risk taking and exploratory activity was evaluated. In line with our first hypothesis, starvation favoured solitariness in sea bass. Conflicting with our second hypothesis, however, food deprivation had no impact on risk taking and exploratory level.

It is important to emphasize that the third component of our PCA, solitariness, explained only 15% of variance in our dataset, suggesting that it should be interpreted with caution. Moreover, because of the methodological approach that we followed, two issues must be outlined regarding how solitariness was assessed in the present work.

First, during the conspecific test, the social stimulus was not visible to the focal fish as it was placed in an opaque jar situated at the centre of the arena. This absence of visual contact between the focal fish and

its conspecific allowed us to interpret the interaction as reflecting true social interest without any establishment of dominant–subordinate relationships. Whether the results would have been the same if the conspecific had been visible or was presented to the focal fish in a different manner (e.g. transparent partition or at a different location in the arena) is open to question. It must be noted, however, that, although not visible, the conspecific inside the jar could still interact with the focal fish through holes that allowed chemical cues and pressure waves to disseminate into the arena.

Second, the possibility exists of a carry-over effect of the risk response to the jar alone (day 2) on that to the presence of a conspecific inside the jar on day 3. For example, fish with reduced interactions with the novel object on day 2 were considered as risk averse and these individuals might have continued to show aversion to this object on day 3, despite the presence of a conspecific inside the jar. Although risk taking was evaluated in the first 5 min of the novel object test (day 2), fish actually remained in the arena for a total of 30 min, familiarizing themselves with the object. It was therefore considered that by day 3, the jar was no longer a novel object, allowing us to consider that interactions with the jar were then fully attributable to the presence of the conspecific inside.

With regard to our first hypothesis, control fish interacted more with conspecifics than did FD fish. Juvenile European sea bass form shoals, a social behaviour that is believed to rely on the fish's voluntary decision to remain together (Réale et al., 2007). Grouping behaviour is increasingly considered as a flexible response by which individuals tune the trade-off between predator avoidance and resource acquisition (Krause et al., 2000b; Lima and Dill, 1990). Shoaling indeed contributes to maximizing food acquisition through increased detection rate and, at the same time, enhances predator avoidance through greater vigilance in addition to dilution and confusion effects. However, grouping behaviour is also associated with stronger competition between conspecifics and easier detection to predators, shoals being more visually conspicuous than solitary individuals (Clifton and Robertson, 1993). The observation that FD fish displayed reduced interactions with the conspecific suggests that when food is scarce, individuals may benefit from solitariness as it reduces competition and aggressiveness prompted by hunger (Webster and Hart, 2006).

Conflicting with our second hypothesis, the experimental results revealed that food deprivation had no impact on risk taking and exploratory activity in sea bass. Individuals from the FD group were initially expected to be risk prone and to display a higher activity level than fish from the control group. It has indeed been hypothesized that a higher level of activity and risk taking favours habitat exploration and consequently enhances food encounter rates (Killen et al., 2011; McFarlane et al., 2004; van Dijk et al., 2002). Moreover, increased activity and risk taking has been reported following starvation (Godin and Crossman, 1994; Killen et al., 2011; McFarlane et al., 2004). To explain this inconsistency between the present results and the literature, differences in experimental design must be considered. While in the present study activity and risk taking were assessed from the viewpoint of novelty, using an unfamiliar environment or a novel object, the studies mentioned above measured spontaneous activity in a familiar environment and assessed risk taking in response to attractive and/or repulsive stimuli. Because of these among-studies discrepancies in experimental design, swimming activity and risk taking were actually evaluated in different contexts in which fish may have expressed a different behavioural response. In the present study, the fact that exploratory activity and risk taking did not change in starved sea bass suggests that after 3 weeks of food deprivation, the trade-off between fulfilling a nutritional requirement and risk taking

in response to novelty was unchanged. It remains to be tested, however, whether this trade-off would have been resolved differently if the fish's energetic status had been aggravated further, by a longer starvation period, for instance.

It should be noted that exploratory activity was nearly statistically different between control and FD fish ( $P=0.057$ ). This suggests that compared with control fish, the exploratory inclination of starved individuals tended to be reduced (Fig. 1B,F). This result is in line with other studies in which decreased activity after food deprivation has been reported (Binner et al., 2008; van Dijk et al., 2002). Swimming is energetically costly and it would make sense that fish with a reduced energy reserve lowered the expression of energetically demanding activities such as swimming. However, lowering exploratory activity also implies reduced chances of finding food. In the present study, no food was made available to the fish, possibly rendering increased swimming inappropriate. Yet, the strategy could have been different if food had been made available, augmenting the potential reward of increased exploratory activity.

### Correlation among behaviours

The observed trade-off between risk taking and solitariness in risk-prone individuals validated our third hypothesis. After 3 weeks of food restriction, we indeed demonstrated that risk-prone FD fish interacted less with conspecifics (increased solitariness) while risk-prone control fish interacted more with conspecifics. As discussed above, food restriction does not affect risk taking. This suggests, therefore, that the origin of the risk taking  $\times$  solitariness interaction in risk-prone fish lies in the modulating effect of fish nutritional status upon solitariness.

Under the control feeding condition, individuals that interacted the most with the novel object also exhibited the highest interest for conspecifics. This result is in accordance with observations reported by Cote and Clobert (2007) on *Lacerta vivipara*, but it contradicts studies in which risk-prone fish were reported to ignore conspecifics and to engage in fewer interactions than risk-averse individuals (Budaev, 1997; Harcourt et al., 2009a; Wilson et al., 1993). Juvenile sea bass are gregarious fish that form shoals (Barnabé, 1980) and the sharing of food is not always equal within a shoal (Bumann and Krause, 1993; Krause, 1993; Ward et al., 2004). It can be hypothesized that fish with a high propensity to take risks also benefit from solitariness to find and secure food without competition. Similarly, Harcourt et al. (2009b) reported that hunger modulated shoaling behaviour differently in shy and bold three-spined sticklebacks. They showed that under normal feeding conditions, shy and bold individuals both preferred to shoal with bold conspecifics. In contrast, under restrictive feeding conditions, bold sticklebacks continued to prefer shoaling with bold fish while shy individuals displayed a preference for shy conspecifics. Harcourt et al. (2009b) hypothesized that the change in shoaling preference observed in the starved shy individuals suggests that these fish perceived more acutely the potential intraspecific competition by the bold fish and responded by shoaling with shy and potentially less competitive conspecifics. This modulation of social behaviour by the interaction between feeding status and risk-taking behaviour is along the same lines as our own results, which indicate that in periods of food shortage, the potential reward associated with taking more risks and being less sociable may overcome the cost resulting from increased predation risk related to solitariness.

To conclude, the present findings document how hunger may affect sociability in juvenile sea bass and contribute to increase current knowledge regarding the interaction between feeding status and risk taking and how it can drive social behaviour.



**Acknowledgements**

We would like to thank the members of the PFOM-ARN laboratory (Ifremer) for their technical assistance. We are also grateful to C. Lebigre for helpful advice on statistical analyses. We also thank the anonymous referees for their helpful comments on the manuscript.

**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**

Conceptualization: C.A., G.C.; Methodology: C.A., N.L.B.; Formal analysis: C.A.; Investigation: C.A.; Writing - original draft: C.A.; Writing - review & editing: C.A., G.C.; Visualization: C.A., G.C.; Supervision: S.L.F., G.C.

**Funding**

C.A. was supported by a joint CEDRE–Région Bretagne doctoral grant.

**Supplementary information**

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.190553.supplemental>

**References**

- Arber, B., Huntingford, F. A. and Crompton, D. W. T. (1995). The effect of hunger and cestode parasitism on the shoaling decisions of small freshwater fish. *J. Fish Biol.* **47**, 524-536.
- Ariyomo, T. O. and Watt, P. J. (2012). The effect of variation in boldness and aggressiveness on the reproductive success of zebrafish. *Anim. Behav.* **83**, 41-46.
- Barnabé, G. (1980). Exposé synoptique des données biologique sur le loup ou bar *Dicentrarchus labrax* (Linné, 1758). *FAO Fish. Synop.* **126**, 1-70.
- Bell, A. M. (2005). Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). *J. Evol. Biol.* **18**, 464-473.
- Bell, A. M. and Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). *Ecol. Lett.* **10**, 828-834.
- Bell, A. M. and Stamps, J. A. (2004). Development of behavioural differences between individuals and populations of sticklebacks, *Gasterosteus aculeatus*. *Anim. Behav.* **68**, 1339-1348.
- Binner, M., Kloas, W. and Hardewig, I. (2008). Energy allocation in juvenile roach and burbot under different temperature and feeding regimes. *Fish Physiol. Biochem.* **34**, 103-116.
- Biro, P. A. and Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends Ecol. Evol.* **25**, 653-659.
- Boulton, K., Grimmer, A. J., Rosenthal, G. G., Walling, C. A. and Wilson, A. J. (2014). How stable are personalities? A multivariate view of behavioural variation over long and short timescales in the sheephead swordtail, *Xiphophorus birchmanni*. *Behav. Ecol. Sociobiol.* **68**, 791-803.
- Brown, C., Jones, F. and Braithwaite, V. (2005). In situ examination of boldness–shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. *Anim. Behav.* **70**, 1003-1009.
- Budaev, S. V. (1997). "Personality" in the guppy (*Poecilia reticulata*): a correlational study of exploratory behavior and social tendency. *J. Comp. Psychol.* **111**, 399-411.
- Bumann, D. and Krause, J. (1993). Front individuals lead in shoals of three-spined sticklebacks (*Gasterosteus aculeatus*) and juvenile roach (*Rutilus rutilus*). *Behaviour* **125**, 189-198.
- Burns, J. G. (2008). The validity of three tests of temperament in guppies (*Poecilia reticulata*). *J. Comp. Psychol.* **122**, 344-356.
- Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlishaw, G. and Heinsohn, R. (2013). Animal personality: what are behavioural ecologists measuring? *Biol. Rev. Camb. Philos. Soc.* **88**, 465-475.
- Chapman, B. B., Hulthén, K., Blomqvist, D. R., Hansson, L.-A., Nilsson, J.-A., Brodersen, J., Anders Nilsson, P., Skov, C. and Brönmark, C. (2011). To boldly go: individual differences in boldness influence migratory tendency. *Ecol. Lett.* **14**, 871-876.
- Cibois, P. (1986). L'analyse des correspondances: l'indispensable retour aux données. *Hist. Amp. Mes.* **1**, 239-247.
- Cibois, P. (1997). Les pièges de l'analyse des correspondances. *Hist. Mes.* **12**, 299-320.
- Claireaux, G., Théron, M., Prineau, M., Dussauze, M., Merlin, F.-X. and Le Floch, S. (2013). Effects of oil exposure and dispersant use upon environmental adaptation performance and fitness in the European sea bass, *Dicentrarchus labrax*. *Aquat. Toxicol.* **130-131**, 160-170.
- Clark, C. W. and Mangel, M. (1986). The evolutionary advantages of group foraging. *Theor. Popul. Biol.* **30**, 45-75.
- Clifton, K. E. and Robertson, D. R. (1993). Risks of alternative mating tactics. *Nature* **366**, 520.
- Coleman, K. and Wilson, D. (1998). Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. *Anim. Behav.* **56**, 927-936.
- Conrad, J. L., Weinersmith, K. L., Brodin, T., Saltz, J. B. and Sih, A. (2011). Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. *J. Fish Biol.* **78**, 395-435.
- Cote, J. and Clobert, J. (2007). Social personalities influence natal dispersal in a lizard. *Proc. R. Soc. B Biol. Sci.* **274**, 383-390.
- Cote, J., Fogarty, S., Weinersmith, K., Brodin, T. and Sih, A. (2010). Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proc. R. Soc. B Biol. Sci.* **277**, 1571-1579.
- Dingemans, N. J., Both, C., Drent, P. J. and Tinbergen, J. M. (2004). Fitness consequences of avian personalities in a fluctuating environment. *Proc. Biol. Sci.* **271**, 847-852.
- Dingemans, N. J., Dochtermann, N. and Wright, J. (2010). A method for exploring the structure of behavioural syndromes to allow formal comparison within and between data sets. *Anim. Behav.* **79**, 439-450.
- Dupont-Prinet, A., Chatain, B., Grima, L., Vandeputte, M., Claireaux, G. and McKenzie, D. J. (2010). Physiological mechanisms underlying a trade-off between growth rate and tolerance of feed deprivation in the European sea bass (*Dicentrarchus labrax*). *J. Exp. Biol.* **213**, 1143-1152.
- Frommen, J. G., Luz, C. and Bakker, T. C. M. (2007). Nutritional state influences shoaling preference for familiars. *Zoology* **110**, 369-376.
- Frost, A. J., Thomson, J. S., Smith, C., Burton, H. C., Davis, B., Watts, P. C. and Sneddon, L. U. (2013). Environmental change alters personality in the rainbow trout, *Oncorhynchus mykiss*. *Anim. Behav.* **85**, 1199-1207.
- Galhardo, L., Vitorino, A. and Oliveira, R. F. (2012). Social familiarity modulates personality trait in a cichlid fish. *Biol. Lett.* **8**, 936-938.
- Gingerich, A. J., Philipp, D. P. and Suski, C. D. (2010). Effects of nutritional status on metabolic rate, exercise and recovery in a freshwater fish. *J. Comp. Physiol. B* **180**, 371-384.
- Godin, J.-G. (1986). Antipredator function of shoaling in teleost fishes: a selective review. *Nat. Can.* **113**, 241-250.
- Godin, J.-G. and Crossman, S. L. (1994). Hunger-dependent predator inspection and foraging behaviours in the threespine stickleback (*Gasterosteus aculeatus*) under predation risk. *Behav. Ecol. Sociobiol.* **34**, 359-366.
- Greenberg, R. and Mettke-Hofmann, C. (2001). Ecological aspects of neophobia and neophilia in birds. In *Current Ornithology*, Vol. 16 (ed. V. Nolan and C. F. Thompson), pp. 119-178. Springer.
- Harcourt, J. L., Ang, T. Z., Sweetman, G., Johnstone, R. A. and Manica, A. (2009a). Social Feedback and the Emergence of Leaders and Followers. *Curr. Biol.* **19**, 248-252.
- Harcourt, J. L., Sweetman, G., Johnstone, R. A. and Manica, A. (2009b). Personality counts: the effect of boldness on shoal choice in three-spined sticklebacks. *Anim. Behav.* **77**, 1501-1505.
- Hensor, E. M. A., Godin, J.-G. J., Hoare, D. J. and Krause, J. (2003). Effects of nutritional state on the shoaling tendency of banded killifish, *Fundulus diaphanus*, in the field. *Anim. Behav.* **65**, 663-669.
- Huntingford, F. A., Andrew, G., Mackenzie, S., Morera, D., Coyle, S. M., Pilarczyk, M. and Kadri, S. (2010). Coping strategies in a strongly schooling fish, the common carp *Cyprinus carpio*. *J. Fish Biol.* **76**, 1576-1591.
- Jolles, J. W., Fleetwood-Wilson, A., Nakayama, S., Stumpe, M. C., Johnstone, R. A. and Manica, A. (2015). The role of social attraction and its link with boldness in the collective movements of three-spined sticklebacks. *Anim. Behav.* **99**, 147-153.
- Kaiser, H. F. (1961). A note on Guttman's lower bound for the number of common factors. *Br. J. Stat. Psychol.* **14**, 1-2.
- Killen, S. S., Marras, S. and McKenzie, D. J. (2011). Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. *J. Anim. Ecol.* **80**, 1024-1033.
- Killen, S. S., Fu, C., Wu, Q., Wang, Y.-X., Fu, S.-J. and Williams, T. (2016). The relationship between metabolic rate and sociability is altered by food deprivation. *Funct. Ecol.* **30**, 1358-1365.
- Krause, J. (1993). The influence of hunger on shoal size choice by three-spined sticklebacks, *Gasterosteus aculeatus*. *J. Fish Biol.* **43**, 775-780.
- Krause, J. and Ruxton, G. (2002). *Living in Groups*. Oxford Series in Ecology and Evolution (ed. P. H. Harvey and R. M. May). New York: Oxford University Press Inc.
- Krause, J., Hoare, D., Krause, S., Hemelrijk, C. K. and Rubenstein, D. I. (2000a). Leadership in fish shoals. *Fish Fish.* **1**, 82-89.
- Krause, J., Hoare, D. J., Croft, D., Lawrence, J., Ward, A., Ruxton, G. D., Godin, J. G. J. and James, R. (2000b). Fish shoal composition: mechanisms and constraints. *Proc. R. Soc. B Biol. Sci.* **267**, 2011-2017.
- Leblond, C. and Reeb, S. G. (2006). Individual leadership and boldness in shoals of golden shiners (*Notemigonus crysoleucas*). *Behaviour* **143**, 1263-1280.
- Lima, S. L. and Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* **68**, 619-640.
- McFarlane, W. J., Cubitt, K. F., Williams, H., Rowell, D., Moccia, R., Gosine, R. and McKinley, R. S. (2004). Can feeding status and stress level be assessed by analyzing patterns of muscle activity in free swimming rainbow trout (*Oncorhynchus mykiss* Walbaum)? *Aquaculture* **239**, 467-484.

- Moretz, J. A., Martins, E. P. and Robison, B. D.** (2007). Behavioral syndromes and the evolution of correlated behavior in zebrafish. *Behav. Ecol.* **18**, 556-562.
- Mutzel, A., Dingemanse, N. J., Araya-Ajoy, Y. G. and Kempenaers, B.** (2013). Parental provisioning behaviour plays a key role in linking personality with reproductive success. *Proc. Biol. Sci.* **280**, 20131019.
- Nakayama, S., Harcourt, J. L., Johnstone, R. A. and Manica, A.** (2012). Initiative, personality and leadership in Pairs of foraging fish. *PLoS ONE* **7**, e36606.
- Nelson, J. A. and Claireaux, G.** (2005). Sprint swimming performance of juvenile European sea bass. *Trans. Am. Fish. Soc.* **134**, 1274-1284.
- Patrick, S. C., Pinaud, D. and Weimerskirch, H.** (2017). Boldness predicts an individual's position along an exploration-exploitation foraging trade-off. *J. Anim. Ecol.* **86**, 1257-1268.
- Pitcher, T. J.** (1986). Functions of shoaling behaviour in teleosts. In *The Behaviour of Teleost Fishes* (ed. T. J. Pitcher), pp. 294-337. Springer.
- Pitcher, T. J. and Parrish, J. K.** (1993). Functions of shoaling behaviour in teleosts. In *The Behaviour of Teleost Fishes*, 2nd edition (ed. T. J. Pitcher). Springer.
- Pulliam, H. R. and Caraco, T.** (1984). Living in groups: is there an optimal group size? In *Behavioural Ecology: An Evolutionary Approach* (ed. J. R. Krebs and N. B. Davies), pp. 122-147. Wiley.
- Raubenheimer, D., Simpson, S. J. and Tait, A. H.** (2012). Match and mismatch: conservation physiology, nutritional ecology and the timescales of biological adaptation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **367**, 1628-1646.
- Réale, D., Reader, S. M., Sol, D., McDougall, P. T. and Dingemanse, N. J.** (2007). Integrating animal temperament within ecology and evolution. *Biol. Rev.* **82**, 291-318.
- Roche, D. G., Careau, V. and Binning, S. A.** (2016). Demystifying animal 'personality' (or not): why individual variation matters to experimental biologists. *J. Exp. Biol.* **219**, 3832-3843.
- Rudin, F. S. and Briffa, M.** (2012). Is boldness a resource-holding potential trait? Fighting prowess and changes in startle response in the sea anemone, *Actinia equina*. *Proc. Biol. Sci.* **279**, 1904-1910.
- Sneddon, L. U.** (2003). The bold and the shy: individual differences in rainbow trout. *J. Fish Biol.* **62**, 971-975.
- Toms, C. N., Echevarria, D. J. and Jouandot, D. J.** (2010). A methodological review of personality-related studies in Fish: focus on the shy-bold axis of behavior. *Int. J. Comp. Psychol.* **23**, 1-25.
- van Dijk, P., Staaks, G. and Hardewig, I.** (2002). The effect of fasting and refeeding on temperature preference, activity and growth of roach, *Rutilus rutilus*. *Oecologia* **130**, 496-504.
- Ward, A. J. W., Thomas, P., Hart, P. J. B. and Krause, J.** (2004). Correlates of boldness in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behav. Ecol. Sociobiol.* **55**, 561-568.
- Webster, M. M. and Hart, P. J. B.** (2006). Kleptoparasitic prey competition in shoaling fish: effects of familiarity and prey distribution. *Behav. Ecol.* **17**, 959-964.
- Wilson, D. S., Coleman, K., Clark, A. B. and Biederman, L.** (1993). Shy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): an ecological study of a psychological trait. *J. Comp. Psychol.* **107**, 250-260.
- Wright, D., Rimmer, L. B., Pritchard, V. L., Krause, J. and Butlin, R. K.** (2003). Inter and intra-population variation in shoaling and boldness in the zebrafish (*Danio rerio*). *Naturwissenschaften* **90**, 374-377.