

Influences of divergent behavioral strategies upon risk allocation in juvenile flatfishes

Kate S. Boersma · Clifford H. Ryer · Thomas P. Hurst · Selina S. Heppell

Received: 28 January 2008 / Revised: 25 June 2008 / Accepted: 8 July 2008 / Published online: 29 July 2008
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Abstract Animals balance feeding and anti-predator behaviors at various temporal scales. When risk is infrequent or brief, prey can postpone feeding in the short term and temporally allocate feeding behavior to less risky periods. If risk is frequent or lengthy, however, prey must eventually resume feeding to avoid fitness consequences. Species may exhibit different behavioral strategies, depending on the fitness tradeoffs that exist in their environment or across their life histories. North Pacific flatfishes that share juvenile rearing habitat exhibit a variety of responses to predation risk, but their response to risk frequency has not been examined. We observed the feeding and anti-predator behaviors of young-of-the-year English sole (*Parophrys vetulus*), northern rock sole (*Lepidopsetta polyxystra*), and Pacific halibut (*Hippoglossus stenolepis*)—three species that exhibit divergent anti-predator strategies—following exposure to three levels of predation risk: no risk, infrequent (two exposures/day), and frequent (five exposures/day). The English sole responded to the frequent risk treatment with

higher feeding rates than during infrequent risk, following a pattern of behavioral response that is predicted by the risk allocation hypothesis; rock sole and halibut did not follow the predicted pattern, but this may be due to the limited range of treatments. Our observations of unique anti-predator strategies, along with differences in foraging and species-specific ecologies, suggest divergent trajectories of risk allocation for the three species.

Keywords Foraging · Predation · Risk allocation · Pleuronectid

Foraging behavior increases the risk of predation both through increased conspicuousness and decreased vigilance (Dill 1987; Lima and Dill 1990), and thus organisms typically restrict foraging when risk is high. Experiments which address the tradeoff between risk avoidance and foraging frequently consider only the brief temporal period surrounding the decision point for the organism—e.g., to feed or not to feed—and do not take into account an animal's prior experience regarding the prevalence of predation risk. However, the distribution of risk varies both spatially and temporally in ecological systems, and therefore organisms should be expected to respond to variation in risk by adjusting their feeding rate, conspicuousness, or forage timing to minimize mortality (Lima 1998).

Energy allocation processes require individuals to weigh the costs and benefits of foraging and anti-predator behavior (Priede 1985; Hurst and Conover 2003). The amplified “duck-and-cover” response to predation risk that is often observed in traditional laboratory experiments cannot be a successful long-term strategy for organisms confronted with frequent or lengthy predator exposure simply because the energetic deficit associated with

Communicated by K. Lindström

K. S. Boersma · S. S. Heppell
Department of Fisheries and Wildlife,
Oregon State University,
Corvallis, OR, USA

K. S. Boersma · C. H. Ryer · T. P. Hurst
Fisheries Behavioral Ecology Program,
Alaska Fisheries Science Center, NOAA—NMFS,
Hatfield Marine Science Center,
Newport, OR, USA

K. S. Boersma (✉)
Department of Zoology, Oregon State University,
Cordley Hall 3029,
Corvallis, OR 97331, USA
e-mail: kate.boersma@science.oregonstate.edu

extended periods of reduced foraging would result in significant fitness costs (Lima 1998). In natural situations, animals may use accrued information on the availability of food resources and the frequency or duration of high-risk periods to modify their foraging behavior, in an attempt to maximize the ratio of food intake to predation risk.

Recently, there has been a renewed focus in the literature on the tradeoffs between anti-predator and foraging behaviors, with an emphasis on how temporal patterns in risk affect the response of prey organisms (Sih and McCarthy 2002; Van Buskirk et al. 2002; Pecor and Hazlett 2003; Laurila et al. 2004; Sundell et al. 2004; Vainikka et al. 2005; Mirza et al. 2006). These studies emerged in response to Lima and Bednekoff's (1999) risk allocation hypothesis (RAH), which suggests that individuals choose behaviors using information they have accumulated on the overall pattern of risk, in particular, the frequency and duration of risk events and the ratio of safe periods to risky periods. Organisms exposed to infrequent, high-risk events are expected to exhibit the greatest anti-predator response during risk, and consequently the least feeding. As risky periods increase in frequency or duration, animals may respond by decreasing anti-predator behavior during both risky and safe periods. Although the RAH is intuitively appealing, the studies that followed showed only weak support, and imply that great variability in prey response exists.

Theoretical research suggests that behavioral strategy can strongly influence how animals perceive, interpret, and respond to risk (Blumstein and Bouskila 1996; Sih et al. 2004), and thus variation in behavioral strategy may be associated with different responses to temporal risk patterns. Even closely related, morphologically similar species can exhibit contrasting behavioral strategies in response to risk (Pecor and Hazlett 2006). A multi-species examination of responses to temporal variation in risk may shed light on the role of risk history in the risk response and the relationship between species-specific ecologies and anti-predator behaviors.

Juvenile flatfishes provide ideal experimental animals for an investigation of the range of responses to temporal patterns of risk because closely related species inhabit different ecological roles and employ contrasting anti-predator behavioral strategies (Table 1). While flatfishes

as a group rely on detection minimization as their primary anti-predator tactic, including crypsis, burial, low body posture, and low activity, a recent work by Lemke and Ryer (2006b) suggests that juvenile English sole (*Parophrys vetulus*), northern rock sole (*Lepidopsetta polyxystra*), and Pacific halibut (*Hippoglossus stenolepis*) each exhibits modifications to this shared cryptic strategy. English sole are risk-prone, choosing conspicuous behaviors regardless of predator presence; northern rock sole are risk-averse, remaining cryptic in both the presence and absence of predators; and halibut are risk-sensitive, modifying behavior in response to perceived risk level. Although all three species inhabit nearshore nursery areas in the North Pacific in their first year post-settlement, English sole preferentially settle in estuaries, while juvenile Pacific halibut and northern rock sole are more frequently found in shallow coastal embayments (Norcross et al. 1997; Hurst et al. 2007). These distinct environments and the associated differences in behavioral strategy provide an opportunity to study the interplay between foraging behavior and anti-predator behavior and the impact of temporal variation in risk on the risk response for closely related, morphologically similar species.

In this study, we examine the tradeoffs between foraging behavior and anti-predator behavior for juvenile English sole, Pacific halibut and northern rock sole exposed to different risk frequencies. Specifically, we hypothesize that over the course of a 5-day trial the divergent anti-predator strategies employed by these species will impact feeding behavior and conspicuousness and will result in different responses to temporal patterns in risk. As a "risk-prone" species (Lemke and Ryer 2006b), we expect that English sole will exhibit high feeding and conspicuousness throughout the trials and that feeding behavior will differ minimally between the risk treatments. The "risk-sensitive" Pacific halibut will demonstrate behaviors that support the risk allocation hypothesis by exhibiting higher feeding and conspicuousness in treatments with more frequent risk than in treatments with less frequent risk. The "risk-averse" nature of northern rock sole will lead to minimal feeding and reduced conspicuousness in all treatments for this species. We test these predictions by manipulating risk frequency and quantifying subsequent feeding and anti-predator behaviors.

Table 1 Species-specific ecology relevant to behavioral strategy

	English sole	Pacific halibut	Northern rock sole	Source
Behavioral strategy	Risk-prone	Risk-sensitive	Risk-averse	(Lemke and Ryer 2006b)
Depth	<5 m	5–40 m	5–70 m	(Norcross et al. 1997; Hurst et al. 2007)
Period of maximum gut fullness	Morning	Afternoon	Evening	(Hurst et al. 2007)
Predation vulnerability	High	Intermediate	Low	(Lemke and Ryer 2006a)
Primary escape tactic	Shallow, turbid water as cover	Flight	Crypsis	(Lemke and Ryer 2006b)

Materials and methods

Collection and maintenance

Age-0 (young-of-the-year) northern rock sole and Pacific halibut (40–60 mm total length) were collected in the coastal waters (5–25 m depth) of Kodiak Island, Alaska, USA in August 2006 using a 2-m beam trawl with 3-mm mesh codend. Fish were held at the National Marine Fisheries Service Kodiak Laboratory for 2–4 days and then shipped to Hatfield Marine Science Center in Newport, Oregon, USA. Fish were air-transported in plastic bags with seawater and oxygen. Duration of transport was approximately 30 h, during which time ice packs were used to maintain cold water temperatures. Upon arrival, fish were transferred to holding tanks (1.22 m in diameter) and later to 2.3-m tanks. Age-0 English sole (40–60 mm total length) were collected in May and June 2006 in Yaquina Bay, Oregon (at depths of 3–5 m) with a 2-m otter trawl with 3-mm mesh codend towed at 1–2 knots. Fish were held at Hatfield Marine Science Center in 2.3-m-diameter tanks until their transfer to experimental tanks. These collection and transport procedures have proven effective with minimal mortality for all three species.

Holding and experimental tanks contained flow-through seawater from adjacent Yaquina Bay, maintained at temperatures of 9–10°C. A thin layer of sand (~0.5 cm) was spread on the bottom of the tanks to allow fish to bury. Fish were exposed to a daily cycle of 11:1:11:1 light/dusk/dark/dawn in the laboratory. All juvenile fish were fed a combination of commercially available pellet food (1 mm Biodiet[®], Bio-Oregon) and krill three times weekly to satiation for the first 2 weeks of captivity. After this period, diet transitioned to 1-mm and later 2-mm Biodiet pellets, fed thrice weekly at a ration of 0.0546 g pellets per gram body weight per day (as per Hurst and Abookire 2006). All fish transfers were conducted with StressCoat[®] in transfer water to minimize handling stress. Time in captivity ranged from 3 weeks to 3 months before the commencement of trials. At the conclusion of the trials, rock sole and halibut were transferred to holding tanks for use in future behavioral experiments, while English sole were released to Yaquina Bay.

Experimental apparatus

Risk trials were conducted in six 0.5-m cubic glass tanks with bottom coverage of sand to a depth of 2 cm in a 2:1 mixture of coarse (1 mm) to medium (0.5 mm) quartz particles (as per Lemke and Ryer 2006b). Each experimental tank was positioned adjacent to a predator tank, separated by 6 cm to ensure visual contact between experimental fish and the predator stimulus, while allowing

space for a 4-cm black foam divider between tanks. Each experimental/predator pair of tanks was separated from other pairs by an opaque black vinyl curtain to prevent transmission of visual cues between treatments. Additional visual barriers were employed to separate the observer from the tank area and maintain daytime experimental light levels at $\sim 10^{-1}$ μmol photons per second per square meter. Video cameras were situated 0.91 m from experimental tanks and at a 20°-angle above the level of the sand to provide an oblique camera view of each tank. Preliminary studies indicated that this view was most efficient at capturing juvenile fish behavior.

The risk treatment was a model predator, a 34.3 cm \times 16.5 cm weighted rubber flounder suspended by transparent line and made to saltate by an observer for the duration of the exposure period. To initiate each risk exposure period, the opaque foam divider was raised remotely to allow juvenile fish visual contact with the model predator. Use of a model predator ensured more consistent behavior than could be achieved with live predators. Preliminary trials indicated that juvenile flatfish responded to the model by exhibiting characteristic anti-predator behavior, and other laboratory experiments involving model predators support this observation (Ryer and Olla 1998). Additionally, pre-trial observations confirmed that fish did not respond to the movement of the foam divider alone.

Commercial food pellets were delivered by feeding tube to the experimental tanks and fell to the substrate at one of two feeding stations located on the surface of the sand. Continuous water flow through Tygon[®] tubing (1.27 cm inside diameter, 1.91 cm outside diameter, Saint-Gobain Performance Plastics) carried the food, one pellet at a time, to one of the feeding stations. Food appeared at the mouth of each feeding tube and fell down through the water column to a raised mesh grate flush with the sand surface. Unconsumed pellets fell through the mesh grate and became unavailable, thus restricting the time period in which each pellet was accessible to the fish to 3–5 s. This procedure allowed researchers to track minute changes in the willingness of the fish to take risks as the predator treatment was manipulated.

Experimental procedures

One week prior to trials, 30 conspecific fish were transferred from holding tanks to a 2.3-m-diameter feeder-acclimation tank in which individuals acclimated to the food delivery system (Fig. 1). Two hours prior to their addition to experimental tanks, the 30 fish were moved from the feeder acclimation tank to a 2.3-m predator-acclimation tank. It is well documented that animals in captivity lose their responsiveness to predatory threat with increasing time in captivity. Time in captivity ranged from

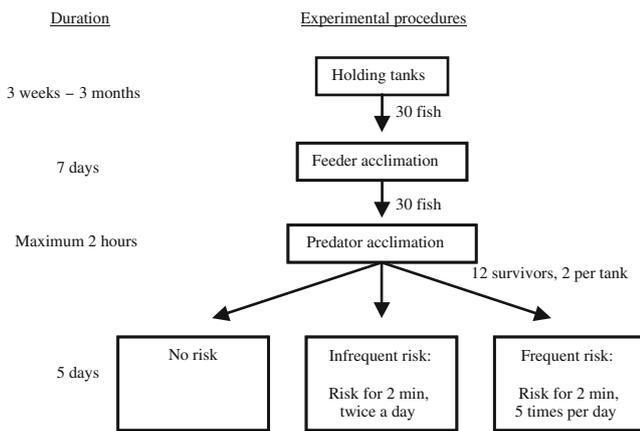


Fig. 1 Experimental procedures and duration of pre-trial acclimation periods. Once an acclimated batch of animals was randomly assigned to the no-risk, infrequent-risk, and frequent-risk treatments to begin the 5-day trial, a new batch of 30 individuals was transferred from the holding tanks to the feeder acclimation tanks. These procedures were repeated four times for each species

3 weeks to 3 months; therefore, the predator acclimation was designed to reinforce the credibility of predation risk after variable periods in captivity for experimental animals. The predator tank contained two age-3 halibut predators (as per Lemke and Ryer 2006b) and exposed juvenile fish to live predation risk. Predators had been deprived of food for 48 h to achieve reliable feeding performance. To minimize mortality, juveniles remained in the predator tank for 2 h or until visual estimation indicated 50% mortality had been reached, whichever was shorter. Mean consumption rates ranged from five to 12 fish, depending on the species. While it is likely that the predator acclimation period selected for animals that had well-developed anti-predator defenses, it was necessary in order to ensure the responsiveness to predatory threat (see Lemke and Ryer 2006a for description of relative vulnerabilities of the three species). Twelve of the surviving fish were relocated to experimental tanks, two fish per tank, and allowed to acclimate before trials began. After introduction to the experimental tanks from the predator exposure tank, trials commenced once fish took food from the feeder system immediately following food introduction (72–96 h). Fish were fed 48 h prior to trials and every 48 h thereafter to ensure equivalent hunger and activity levels (Stoner and Ottmar 2003).

Fish were exposed to three risk treatments: zero-risk exposure (no risk), risk exposure for 2 min twice a day (infrequent risk), and risk exposure for 2 min five times per day (frequent risk). These treatments were determined through extensive preliminary trials with the three species that indicated that 2-min risk periods were sufficient to trigger stereotypic anti-predator behaviors. The same 12 fish remained in experimental tanks for a 5-day trial. The three risk treatments were randomly assigned to the six

experimental tanks, producing two replicates of each treatment per each 5-day trial. Feeding and observation took place on day 1, day 3, and day 5, while risk treatments were applied every day of the 5-day trial so fish did not associate risk treatments with food. The 2-min risk periods were applied at metered intervals between 0900 and 1230 hours, and one of those risk periods, selected at random, was accompanied by a focal period, which included and immediately followed the risk application. The focal period was the period in which the day's ration of food pellets was introduced and coincided with video recording and observation from behind a blind. During each 18-min focal period, risk exposure occurred during minutes 1–2, whereas minutes 3–18 were risk-free. Food delivery during the focal period involved remotely adding three to five pellets (depending on the daily ration) every 1 min for 18 min to alternating feeders, and observation with both video recording and an observer behind the blind. Each experiment was repeated four times, yielding a total of eight replicates of each treatment for each species (Fig. 1).

Data collection

Conspicuousness

We recorded three measures of conspicuousness: posture, burial, and activity level, all of which have been used successfully in other studies as measures of risk-taking behavior in flatfishes (Lemke and Ryer 2006b). Body posture relative to the sediment surface and percent burial in sand were recorded by live observation, while activity level was determined by video analysis. A body posture score was recorded for the fish every 1 min: 0—under sand surface, 1—flat on sand surface, 2—head lifted off of the sand, 3—back arched with head and anterior part of the body off of the sand, and 4—water column activity (Ryer et al. 2004). Degree of burial was scored every 1 min: 0—no sand on any part of the body, 1—less than 25% of the body covered with sand, 2—25 to 50% of the body covered, 3—50 to 75% of the body covered, 4—greater than 75% of the body covered, and 5—body completely covered in sand (Gibson and Robb 1992).

Activity level was calculated by placing a transparent oblique grid with 24 grid squares over the video image of the sand surface and counting the number of line crossings for each fish per 1-min observation period (as per Lemke and Ryer 2006b). Minutes 1–4, 8, 12, and 16 of each focal period were observed. Fish occasionally left the sediment surface and either swam in the water column or settled on the wall of the tank. These rare behaviors were assumed to be artifacts of the small tank size and thus time periods involving movements off of the sediment were removed from further analysis.

Feeding behavior

Feeding behavior was recorded using two measures, both taken via live observation during the focal period: feeding behavior score and latency to first feeding attempt. The feeding behavior score was recorded every minute of the focal period using an index we developed to track feeding behavior relative to food stimulus: 0—no response to food, 1—posture change in response to food, 2—movement toward feeder, 3—physical contact with the grate, and 4—attempted or successful pellet consumption.

Latency to first feeding attempt was calculated as the time in seconds from the appearance of the first pellet at the mouth of the feeding tube until an individual's first feeding strike. Feeding attempt was used as the behavior of choice instead of successful pellet consumption because we assumed that the risk involved in a feeding attempt was equal to that of a successful consumption.

Statistical analysis

Our response variables (i.e., posture, burial, feeding behavior, activity, and latency) were averaged across each pair of fish for each minute. As in previous laboratory experiments (Lemke and Ryer 2006b), fish occasionally exhibited flight responses during the predator exposure period, which increased the variability of the behavior scores during the first few minutes of each focal period. Examination of graphs for each of the coded response variables (posture, burial, and feeding behavior score) by minute and activity level by minute suggested that this response was limited to the first 5 min. To reduce the effects of the variability generated by the flight response, composite scores for each replicate were calculated by averaging the minute-by-minute scores for minutes 5 through 18 of each focal period (minutes 8, 12, and 16 for activity).

The effects of species, treatment, and day upon the posture, burial, feeding behavior, and latency to first feeding attempt were analyzed using repeated measures analysis of variance (ANOVA) and multiple comparisons procedures. Although the variance structures were not equivalent for the three species, the repeated measures ANOVA procedure is robust to minor deviations in variance (Sokal and Rohlf 1981). Multiple comparisons were conducted using Tukey's honestly significant differences (HSD) at the 0.05 significance level.

Due to experimental error and equipment malfunction, there were several missing values in the dataset. We estimated values for these missing cells using the method of minimum mean square error as described by Gill (1978). Degrees of freedom for the analysis were adjusted to reflect only observed data points. The activity level data contained

too many missing values to permit use of the Gill method to fill missing cells and allow for analysis with repeated measures ANOVA. Instead, two-way ANOVA were calculated for each day separately using replicates as nested factors within each treatment.

Results

Autocorrelation among posture and burial was significant (Pearson correlations: posture/burial $r_{216}=-0.92$, posture/feeding behavior $r_{216}=0.77$, feeding behavior/burial $r_{216}=0.67$), therefore posture was chosen as a representative variable of interest to assess conspicuousness. Similarly, graphical exploration revealed that autocorrelation was present for the feeding behavior indices; therefore, the primary analysis was completed on latency to first feeding attempt. The other behavioral metrics revealed similar patterns.

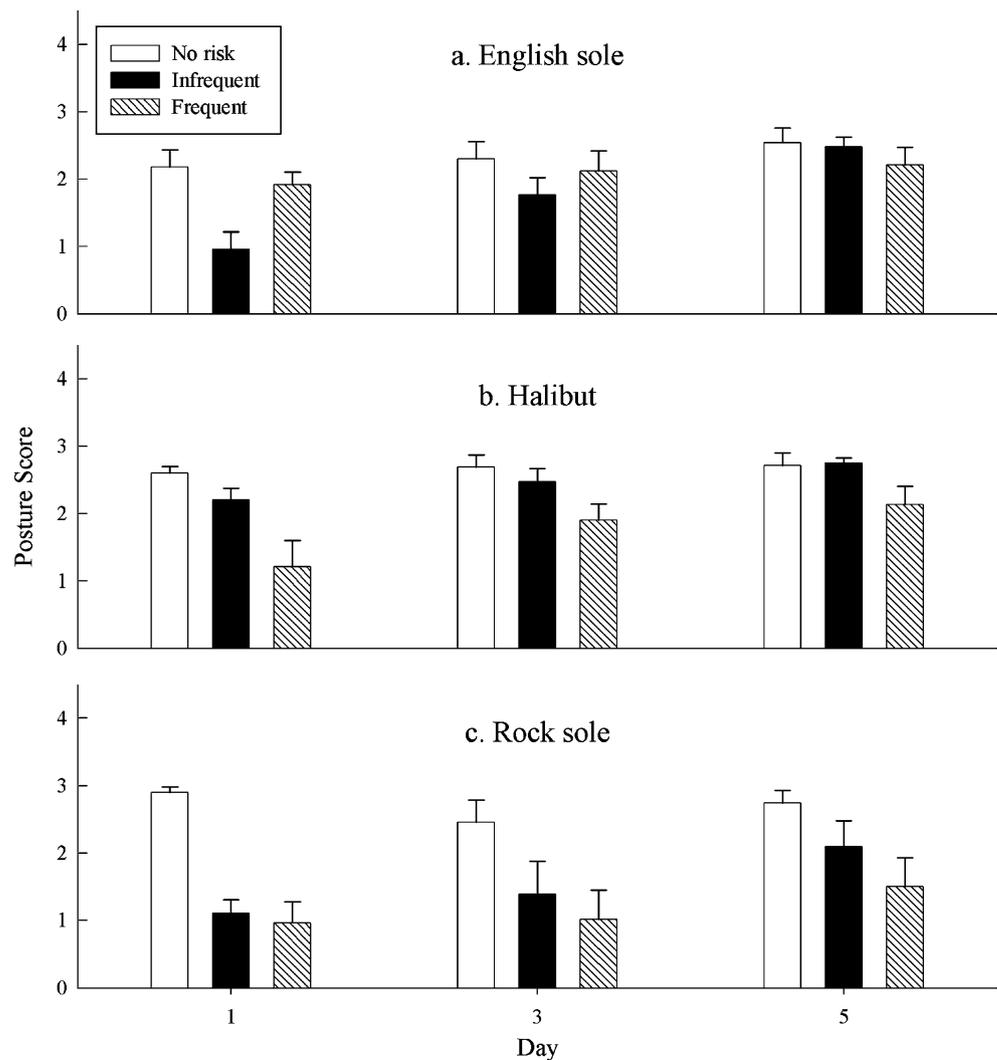
Conspicuousness

The frequency of risk affected the tradeoff between foraging and anti-predator behavior, and these effects varied by both species and day of the week (Fig. 2). The posture scores show that English sole, rock sole, and Pacific halibut responded differently to the three risk treatments (ANOVA: treatment \times species interaction, $F_{4, 47}=3.25$, $P=0.020$). Day of the week (1, 3, or 5) also had a significant effect on how risk treatment affected posture (ANOVA: treatment \times day interaction, $F_{4, 110}=4.13$, $P=0.004$); however, this response was not species-specific (ANOVA: species \times day interaction, $F_{4, 110}=1.24$, $P=0.30$), suggesting that all three species increased conspicuousness similarly as the trial progressed.

Species-specific repeated measures ANOVA and Tukey's HSD multiple comparison procedures were used to detect differences between groups (Tukey correction, $P<0.05$). Only English sole exhibited higher feeding behavior when exposed to frequent risk than infrequent risk, and only on the first day of the trial (Fig. 2a). Day 1 posture scores for the no-risk and frequent-risk treatments for this species were significantly higher than those observed in the infrequent treatment, while no difference was detected between the no-risk and frequent-risk treatments. English sole posture increased over the course of the week in all three treatments, but to the greatest degree in the infrequent treatment, in which the posture scores increased significantly between day 1 and day 3, and between day 3 and day 5.

In contrast to English sole, the posture scores of Pacific halibut decreased with increasing risk frequency (Fig. 2b). On day 1, posture differed significantly between the no-risk treatment and the frequent treatment, reflecting a move to

Fig. 2 Mean \pm SE posture scores by day for English sole, halibut, and rock sole exposed to no risk, infrequent risk, or frequent risk over a 5-day trial. Posture scores ranged from 0 to 4, with 0 as the least conspicuous (flat under the sediment) and 4 as the most conspicuous (water column swimming). $N=61$, 70, and 69 for English sole, halibut, and rock sole, respectively



more conservative behavior with increasing frequency of risk. The posture score at infrequent risk was intermediate between those observed at no risk and at frequent risk.

Rock sole behaved conservatively in both treatments involving risk (Fig. 2c). On day 1, no difference in posture was observed between the infrequent and frequent treatments. However, the difference between the no-risk treatment and either the infrequent or the frequent treatment was significant.

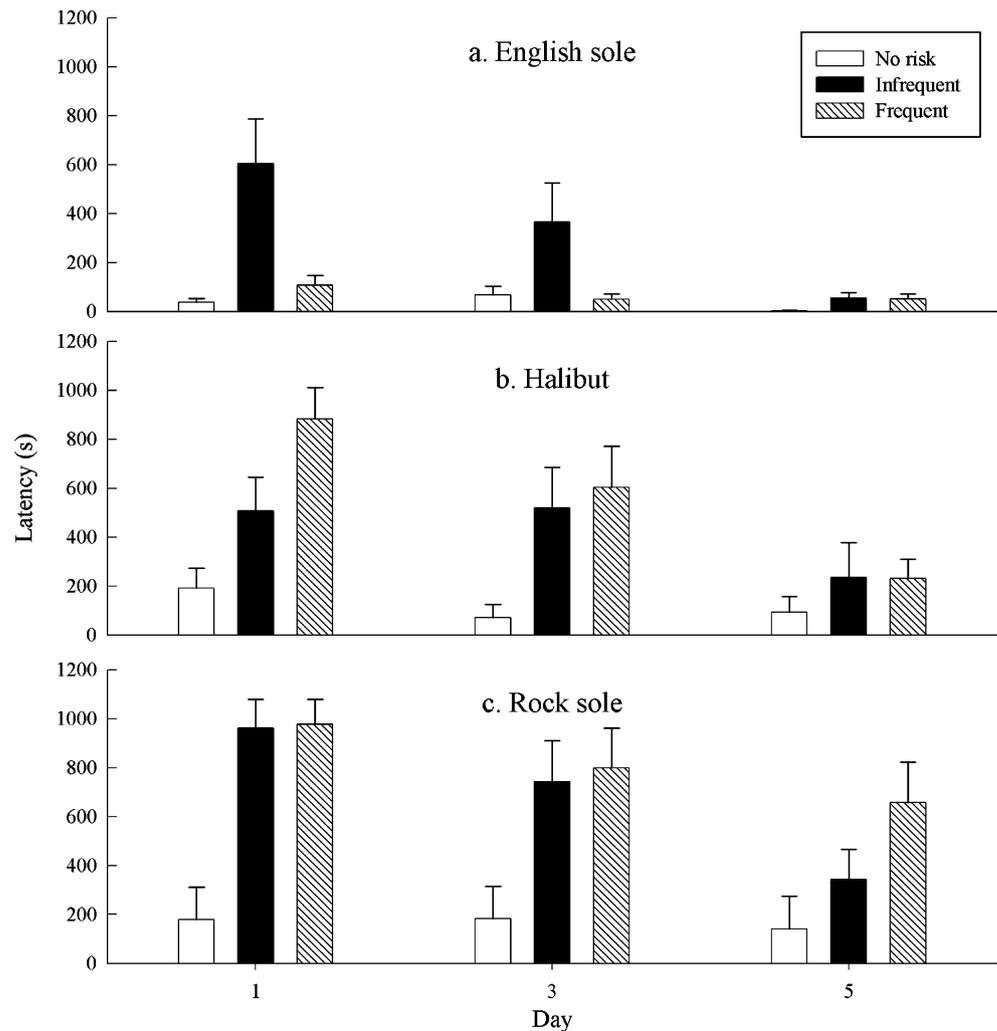
Feeding behavior

The latency to first feeding attempt was affected by risk frequency, species, and day over the course of the trial (Fig. 3). The way in which the risk treatments affected fish feeding varied by species (ANOVA: treatment \times species interaction, $F_{4, 47}=3.63$, $P=0.012$) and by day (ANOVA: treatment \times day interaction, $F_{4, 109}=4.90$, $P=0.001$). However, the species by day interaction and three-way interaction were insignificant (ANOVA: species \times day interaction, $F_{4, 109}=$

0.57 , $P=0.68$; species \times treatment \times day interaction, $F_{8, 109}=1.59$, $P=0.14$).

We ran repeated measures ANOVA on each of the species separately and used Tukey's HSD multiple comparison procedures to detect differences between groups (Tukey correction, $P<0.05$). Species-specific differences in latency confirmed our observations from the posture scores (Fig. 3). English sole postponed feeding to a greater degree during infrequent risk than frequent risk. On day 1, the infrequent treatment was significantly different from either the no-risk or frequent-risk treatments; however, the no-risk and frequent-risk treatments did not differ from each other. On day 1, halibut latency increased with the frequency of risk: the no-risk treatment was significantly different from the frequent-risk treatment, but the infrequent-risk treatment was not different from either the no-risk or frequent-risk treatments. Rock sole latencies to feed were very high in both infrequent and frequent-risk treatments on day 1, and latency in both risk treatments differed significantly from the no-risk

Fig. 3 Mean \pm SE latency scores for English sole, halibut and rock sole exposed to no risk, infrequent risk, or frequent risk over a 5-day trial. Latency was the time period in seconds between the appearance of the first food pellet at the mouth of the feeding tube and an animal's first feeding strike. The focal period was 18 min in duration, so the maximum possible latency for each tank was 1080 s. $N=61, 69,$ and 69 for English sole, halibut, and rock sole, respectively



treatment. As the week progressed, differences between treatment groups for all species disappeared (Fig. 3).

Analysis of activity level also revealed species- and treatment-specific differences. On day 1, activity level varied by treatment (ANOVA: $F_{2, 73}=10.62, P=0.0001$). By day 3, there was evidence of a treatment effect (ANOVA: $F_{2, 76}=10.62, P=0.0029$) and a species effect (ANOVA: $F_{2, 76}=10.62, P=0.036$), but by day 5, there was no effect of treatment or species upon activity level (ANOVA: treatment, $F_{2, 48}=1.15, P=0.33$; species, $F_{2, 48}=1.21, P=0.31$). The conclusions drawn from activity level data generally paralleled those obtained from the posture scores, suggesting consistency between these two conspicuousness measurements.

We also examined feeding behavior and burial, and both response variables showed similar species-specific differences as were observed for posture, latency, and activity level. The effect of risk treatment on both feeding behavior and burial varied by species (ANOVA: feeding behavior, species \times treatment interaction, $F_{4, 47}=4.52, P=0.0036$; burial, species \times treatment interaction, $F_{4, 47}=3.52, P=$

0.014), and both were affected by day (ANOVA: feeding behavior, treatment \times day interaction, $F_{4, 110}=2.61, P=0.039$; burial, day, $F_{2, 110}=8.05, P=0.00055$).

Discussion

Risk frequency affected the three species of juvenile flatfish differently, although generally not in the manner we had predicted. English sole was the only species that followed the behavior pattern predicted by the RAH (Lima and Bednekoff 1999) by responding to increasing risk frequency with the resumption of feeding behavior. These fish had a more marked reduction in feeding and conspicuousness in the infrequent-risk treatment than in the frequent-risk treatment. We predicted that halibut would respond to the frequent-risk treatment by increasing foraging and conspicuousness when compared with the infrequent-risk treatment. Instead, halibut scaled their behavioral response to parallel the frequency of risk, reducing feeding behavior and conspicuousness drastically during the frequent-risk

treatment and to a lesser degree during the infrequent-risk treatment. As we predicted, rock sole did not differentiate between the infrequent and frequent-risk treatments and behaved conservatively in both.

Although the RAH may account for the high feeding and conspicuousness observed with increasing risk frequency for English sole, the halibut and rock sole displayed behaviors that were not consistent with this hypothesis. We believe that their divergent behavioral strategies correspond to species-specific ecologies (Table 1), which affect the tradeoff between anti-predator behavior and foraging behavior and determine the trajectory of risk allocation.

Of the three species, English sole conformed best to a pattern of behavior that would be predicted by the RAH, in which increasing risk frequency leads to an eventual relaxation of anti-predator behavior and resumption of feeding behavior (Lima and Bednekoff 1999). Furthermore, English sole modified their behavior on day 1 of the trial, suggesting that they have both the perceptive ability and the behavioral plasticity to detect and respond rapidly to changes in risk frequency (Figs. 2, and 3). Predation vulnerability experiments suggest that English sole are consumed at greater rates than either the halibut or the rock sole (Lemke and Ryer 2006a; Ryer et al. 2008). English sole may balance safety and foraging by their use of shallow nursery areas, at depths of less than 5 m, which afford protection from predators (Norcross et al. 1997; Hurst et al. 2007). Additionally, Lemke and Ryer (2006a) suggest that the high turbidity associated with their shallow water habitat may promote the persistence of a relaxed anti-predator strategy.

Predation trials with juvenile English sole in varying turbidity regimes demonstrated that consumption rates by live predators decreased significantly under levels of turbidity equivalent to those found in juvenile nursery areas when compared with clear water trials (Lemke and Ryer 2006b). Despite high vulnerability to predators, the low predator densities and high turbidity that characterize English sole's preferred habitat allow juveniles to maintain high conspicuousness, recover rapidly from predation events, and incorporate knowledge of risk history into subsequent behavioral decision-making.

Halibut scaled their anti-predator and feeding behavior to match the frequency of predation risk, behaving conservatively in the frequent treatment and less so during the infrequent treatment (Figs. 2 and 3). This unexpected behavior might be explained by constraints associated with their behavioral strategy and nursery habitat. Juvenile Pacific halibut are found in waters of 5–40 m depth (Norcross et al. 1997; Hurst et al. 2007). It is a well-accepted principle in fisheries biology (Heincke's Law) that the abundance of large predatory fish increases with water

depth (Swain and Morin 1997), and therefore halibut are exposed to greater predation pressure than English sole. These relatively predator-rich waters may cause halibut to delay the resumption of feeding in the face of increasing risk frequency to levels beyond those tested in this experiment, as will be discussed below.

The rock sole exhibited reduced feeding and conspicuousness during both risk treatments, and had not fully recovered feeding behavior by day 5 of the trial. In nursery areas, rock sole inhabit the widest depth range (5–70 m) of the three species and thus are exposed to significant predation pressures. They are also the smallest at the time of summer nursery occupation and therefore the least likely to avoid predation via gape limitations of predators (Hurst et al. 2007). These factors may explain the conservative feeding and anti-predator behavior observed for rock sole in this experiment. The rock sole need not differentiate between risk frequencies for their strategy to be successful, only between the presence and absence of risk. The high

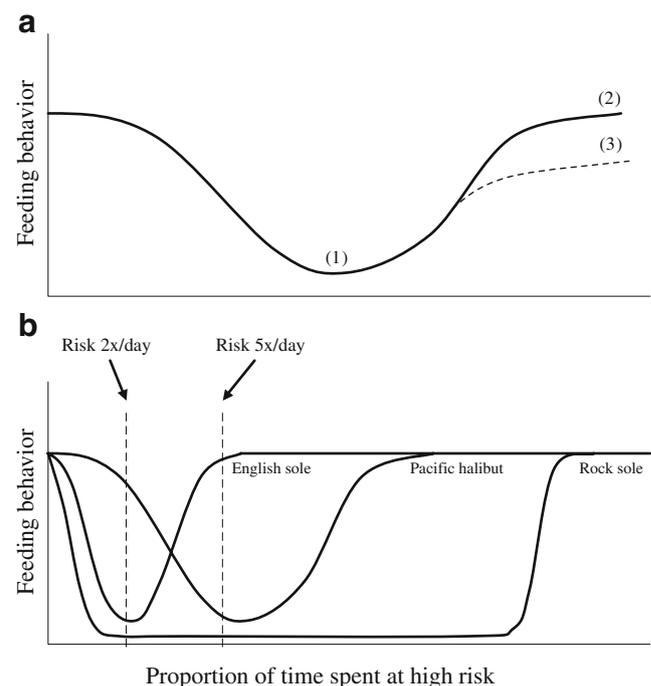


Fig. 4 Conceptual diagram of risk allocation trajectories relative to increasing time spent at high risk. The graph in **a** depicts a stereotypical antipredator response. As the proportion of time spent at high risk increases, feeding behavior decreases until a threshold (1) is reached. After the threshold has been passed, feeding behavior will resume, despite further increases in the proportion of time spent at high risk. Animals may fully recover feeding behavior (2) or only partially (3). The graph in **b** depicts the hypothetical relationship between feeding behavior and the proportion of time spent at high risk for English sole, halibut, and rock sole. The dotted lines represent the risk frequencies tested in this experiment. English sole required a lower proportion of time spent at high risk to demonstrate behavioral compensation than either halibut or rock sole

selective pressure of their predator-rich environment may encourage conservative behavior.

A more holistic way to explain the observed differences in behavioral responses is through an analysis of species-specific trajectories of risk allocation. For example, Fig. 4a represents a typical anti-predator response. As risk becomes more frequent or lengthy (as the proportion of time spent at high risk increases), feeding behavior decreases. But after a threshold, despite increasing risk, energetic demands necessitate the resumption of feeding behavior and the achievement of full or partial behavioral compensation (Lima and Bednekoff 1999). This threshold is likely determined by a number of factors, including individual condition, perceived pattern of risk, the ratio of risky periods to safe periods, and temporal periodicity of food resources (Blumstein and Bouskila 1996; Lima and Steury 2005).

Each species may require different frequencies of risk to reach the threshold and thus the curve may take on different trajectories, though all should be expected to follow a similar general pattern, given an increasing ratio of risky periods to safe periods. Fig. 4b depicts hypothetical curves for English sole, halibut, and rock sole based on the results of this experiment. English sole exhibited complete recovery of feeding behavior in the frequent treatment on day 1, whereas more time and risk frequency were required to trigger even minor recovery of feeding behavior in halibut and rock sole. At different scales of risk exposure per unit time, full behavioral compensation may occur for the halibut and the rock sole as well. It is possible that all three species could exhibit behaviors that are consistent with the RAH if given appropriate experimental conditions.

Risk allocation trajectories may explain the discrepancies between the results of this experiment and those of previous behavioral experiments with English sole. Lemke and Ryer (2006b; 2006a) found no response of juvenile English sole to predator presence, whereas in this study English sole was the only species to demonstrate the suppression and recovery of feeding with increasing risk frequency as predicted by the RAH. We suggest that the predator stimulus used in previous behavioral studies with this species may have exceeded the threshold for behavioral compensation. Based on the results of this study, it is not surprising that many of the attempts to test the RAH have failed to provide convincing empirical support (Laurila et al. 2004; Sundell et al. 2004; Vainikka et al. 2005; Mirza et al. 2006; Slos and Stoks 2006). Behavior must be examined as one component of a larger picture that incorporates species- and population-specific information to understand the behavioral decision-making process. Additionally, the distinct trajectories of risk allocation may be new components of anti-predator strategies that should be considered in behavioral studies.

Some authors have highlighted the difficulty in assessing whether changes in anti-predator responses over time represent risk allocation or laboratory animals' habituation to risk (Hamilton and Heithaus 2001; Brown et al. 2006). In this study, the conspicuousness and feeding indices increased over the course of the week for all three species. Therefore, when the data were viewed over the 5-day trial, it was impossible to distinguish whether the fish were adapting to different risk frequencies or simply becoming aware that the predator posed no significant risk. However, when day 1 data were viewed in isolation, it appeared that juveniles were able to incorporate knowledge of risk history even with very little experience with the model predator. We suggest that the day 1 behaviors may provide the best snapshot of the effects of the RAH with minimal interference from habituation effects. Further experiments involving greater observation time on day 1 could clarify the relationship between risk allocation and habituation and allow for more specific conclusions about the manner in which each species incorporates environmental information into the behavioral decision-making process.

Additionally, it is likely that the predator acclimation period used in this study selected for animals with well-developed anti-predator defenses. Studies that examine a broader range of risk frequencies and involve animals with less variability in holding time are necessary in order to assess the generality of the observed behavioral differences. An examination of behavioral response in the presence or absence of a predator-acclimation period may also be desirable in order to assess differences in learning between species.

As the field of behavioral ecology moves away from single-species, stimulus/response experiments toward more complex multi-factor studies, species-specific ecology and trajectories of risk allocation may facilitate interpretation of experimental results. Different scales of predator exposure may be warranted in order to achieve equivalent risk treatments in multi-species experiments. Although it is commonly believed that all flatfish exhibit similar behaviors as juveniles, we found that species-specific differences in behavioral strategy were strong enough to affect responses to temporal variation in risk. This may necessitate a reevaluation of how theoretical assessments of anti-predator behaviors are conducted.

Acknowledgments We thank B. Laurel and two anonymous reviewers for their constructive comments on this manuscript. We are indebted to S. Haines, P. Iseri, J. Lemke, and M. Ottmar for laboratory and field assistance, and A. Abookire, E. Munk, M. Spencer, and T. Tripp for their help with fish collection. We also thank the many volunteers who assisted with fish collection in Yaquina Bay. This study was conducted as a component of the M.S. research of K.S.B. at Oregon State University. The work was funded by the North Pacific Research Board, grant #R0301 to C. Ryer, A. Abookire, I. Fleming, and A. Stoner. Additional assistance was provided to K.S.B.

by Hatfield Marine Science Center's Markham Scholarship. Experiments were conducted in accordance with the animal care protocols established by the National Marine Fisheries Service, Fisheries Behavioral Ecology Program, and all experiments complied with the current laws of the USA.

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