

## Testing the potential effects of shellfish farming on swimming activity and spatial distribution of sole (*Solea solea*) in a mesocosm

P. Laffargue<sup>a</sup>, M-L. Bégout\*<sup>a</sup> and F. Lagardère<sup>a</sup>

<sup>a</sup>CRELA (UMR 6217 CNRS, Ifremer, University of La Rochelle), BP 5, F-17137 L'Houmeau, France

\* : mlbegout@ifremer.fr

**Abstract:** Restructuring coastal fish nursery habitats by extensive shellfish farming in the French part of the Bay of Biscay could influence fish physiology and behaviour and affect the ecological performance of the species. The influence of oyster-trestle cultivation installations on sole (*Solea solea*) swimming behaviour was investigated using an experimental pond mesocosm. A pen was constructed with three interconnected zones (two with bags of live oysters or oyster shells on trestles, and one free zone). Water renewal depended on the tide. Environmental variables were recorded continuously (temperature, oxygen, pH, meteorological data), every 3–5 days (salinity, samples taken for water analysis and estimation of sedimentation rate) or intermittently (illumination). Sediment cores were taken in each zone before and after the experiment, for sediment redox and water content, plus an evaluation of potential prey. Fish movements (nine sole collected in the wild and tagged with electronic transmitters) were registered during three fortnight-long sessions in spring 2002. In addition to shading from the oyster trestles, water and sediment properties changed significantly in the live oyster zone. Environmental changes and sole swimming behaviour were linked by direct or indirect effects: swimming activity for eight of nine sole followed a diel cycle, with greater travelling distance by night. All the environmental variables modulated swimming activity, but temperature increase, water renewal, and climatic events were associated with key changes between sessions or individual sole. A multilinear regression analysis suggested strongest links with oxygen, atmospheric pressure, light level, water column height, and pH. All sole moved around the enclosure at night, probably to forage. By day, except during gale-force wind, sole returned to the same resting zones. Under the oyster-rearing structures appeared to be the most attractive resting sites. Sole seemingly can occupy an entire nursery in a shellfish farming basin that has moderate habitat changes.

**Keywords:** acoustic telemetry; behavioural responses; environmental constraints; fisheries; flatfish habitat; shellfish farming; tidal pond experiment

## Introduction

Habitat utilization by an organism reflects the spatial distribution of essential resources, the internal state of the organism, and its response to ambient conditions (Huntingford, 1993). By identifying and quantifying habitat preferences, it is possible to evaluate tolerance of an organism to environmental change, and to estimate the essential conditions of its short- and long-term survival. For example, the amount of time juvenile (age 2–3) Atlantic cod spent on different substrata varies according to diel and seasonal cycles (Cote *et al.*, 2003). Habitat alteration by human-induced eutrophication and heavy fishing may therefore influence juvenile abundance, and negative consequences on pelagic and demersal fish populations have been reported from the Black Sea (Daskalov, 2003).

Coastal zones, bays and estuaries contain nursery habitats for numerous flatfish species, including sole (*Solea solea*; Amara *et al.*, 2000; Van der Veer *et al.*, 2001). There, juveniles have to face highly variable hydrodynamic and hydrological conditions, on top of which are superimposed the effects of human activities (Beck *et al.*, 2001). Bays and estuaries along the coast of the Charente-Maritime and Vendée regions of western France are a nursery area for Bay of Biscay sole stocks, especially where the substratum is soft and <5 m deep (Le Pape *et al.*, 2003), and the same regions also host large commercial shellfish operations (mostly for oysters and mussels). The surface area devoted to oyster culture alone is 40 km<sup>2</sup> in the Charentais Straits (“pertuis Charentais”, 45°50’–46°20’N, 1°05’–1°40’W), and it covers 11% of the soft intertidal substrata available (A. Bodoy, pers. comm.). In addition, the oyster cultivation techniques modify the physico-chemical properties of underlying sediments and surrounding waters (Feuillet-Girard *et al.*, 1994). The consequences on benthic fauna include a decrease in species diversity for benthic macrofauna and an increase in the number of species that tolerate conditions of organic enrichment (Nugues *et al.*, 1996). Therefore, shellfish cultivation induces changes in habitat that might affect the settlement of young sole and the establishment of sole nursery areas because both areas lie in the upper intertidal zone (Lê, 1983; Marchand and Masson, 1989). Oyster farming is a major economic activity in the region, and recent research has focused on how both natural and cultivated resources may be protected through sustainable management practice. The present study is part of a larger investigation to evaluate the potential for maintaining flatfish nursery habitats of acceptable quality over the long term in the coastal waters of the Charentais Straits, by defining the surface area of actual functional fish nursery habitats relative to the area of soft substratum potentially available.

In this context, the objective of the present study was to establish whether sole utilize areas where shellfish are cultured, and to identify swimming behaviour variations in relation to environmental modifications that could indicate changes in habitat suitability. The presence of extensive installations of oyster trestles in the shallowest soft substrata of the study region precludes *in situ* trawling for fish, and the turbidity prevents visual observation of the seabed. Therefore, we conducted our study in a salt marsh mesocosm with simulated conditions approximating the natural system, except for hydrodynamic conditions.

## Material and methods

The experimental objectives were: (i) to measure the effects of adding oyster trestles on the habitat, by monitoring hydrological and sediment characteristics, and (ii) to monitor sole swimming activity and habitat utilization with respect to hydrological and climatic changes and oyster-trestle presence using acoustic telemetry. The experiment took place in a saltwater pond (the mesocosm) that had been modified to offer different habitats (Figure 1). One zone was devoted to oyster-trestle culture, a second zone was structure-free, and a third contained

just the physical structures used in oyster cultivation (trestles and shell bags plus shell material).

### Experimental mesocosm

An inlet channel connects the open coastal waters of the Breton Strait to the saltmarsh mesocosm. The pond area is 600 m<sup>2</sup> and was formerly used to culture oysters (46°09'N, 01°09'W), but it is now operated by a research laboratory. Seawater renewal depends on the macrotidal regime, and only takes place for tide ranges >5.5 m (water column height in the pond between 0.8 m and 1.7 m). While preparing the mesocosm for the experiment, walking on and digging into the sediments within the enclosure were avoided to preserve the structure of the surface mud.

A PVC net 2 m high was installed in the pond to enclose a surface area of 200 m<sup>2</sup>, forming three zones of equal surface (56 m<sup>2</sup>) interconnected by an intermediate zone (M-zone; Figure 1). The entire enclosure was at the same depth other than at the borders of the basin and near the inlet pipe. The oyster zone, or O-zone, was designed to replicate the conditions of the oyster-trestle style cultivation technique: eight trestles were installed, each holding six bags containing about 6 kg of oysters (172 ± 18.6 oysters per bag; average individual oyster weight 35.3 ± 4.3 g), totalling 300 kg of oysters. The oyster density was therefore about 200 oysters m<sup>-2</sup> (7 kg m<sup>-2</sup>), approximating the conditions on oyster farms in Marennes-Oléron Bay (Feuillet-Girard *et al.*, 1994). The oyster shell, or S-zone, was similar to the O-zone (8 trestles, 48 bags), except that the same number of oyster shells replaced live oysters in the bags. Therefore, in the S-zone, only the physical features of an oyster production ground were replicated, in particular those attributable to farming structures (shading and settlement of epibionts). The third area was the free zone, or F-zone, and it contained no added structures or shellfish. The pond was filled with seawater on 8 January 2002, and oyster bags were placed onto the trestles on 27 February and 2 March.

### Fish collection and characterization

Sole were caught by trawl from a small fishing boat in the Breton Strait (46°15'N 01°06'W), on 28 February and 1 March. Before handling, the fish were anaesthetized for 3 min (2-phenoxy-ethanol, 0.25 ml l<sup>-1</sup>) in order to be measured for total and standard length ( $L_t$  and  $L_s$ ) and weight ( $W_w$ ). Each sole was implanted with a unique subcutaneous visible alphanumeric tag that could be read under UV light (VI-alpha tag, Northwest Marine Technology, Inc.). Nine sole were selected for tagging with ultrasonic transmitters. To reduce the ratio between tag weight (3.3 g in air, V8SC VEMCO Ltd) and fish weight, the size and weight of the selected fish averaged 23.8 cm (± 0.6)  $L_t$  and 100.8 g (± 9.2)  $W_w$ . The ultrasonic transmitters were externally attached to the fish on the eyed side, transversally through the back. Sole were released into the enclosure 72 h after tagging (30 March; 84 days after the pond was filled).

An additional 13 untagged sole were present in the mesocosm, and nine of these were used as control to test for tag effects (Bégout Anras *et al.*, 2003). Therefore, the initial total biomass was 3 100 g for 22 sole, equivalent to a stocking density of 15 g m<sup>-2</sup>. Of the nine fish tracked, eight were recaptured at the end of the experiment on 24 May; one sole was lost while draining the pond. These eight sole were measured, weighed, their sex determined, and their ages estimated from their otoliths (Table 1). Their health was satisfactory overall, and they had no visible external injuries, particularly with respect to the suture healing around the transmitter attachment. However, the transmitter attachments were often loose, causing some skin lesions caused by friction.

## Acoustic telemetry

A fixed telemetry system (Vemco Radio-linked Acoustic Positioning, Vemco Ltd, Halifax, NS, Canada) detected and recorded the position of fish in the mesocosm. The system consisted of three buoys, each equipped with an omnidirectional hydrophone and a radio-operated modem for transmitting data back to a base station. Each immersed hydrophone was installed at a fixed location (Figure 1), such that the array covered the entire study area. The automated system monitored the frequency of each tagged fish successively for 10 s, consecutively recording the positions for the three sole tracked during each session. The positions were accurate to within 1 m (Klimley *et al.*, 2001). This technique quantified the activity of individual sole in terms of swimming, rhythm, amplitude, and space use.

Recording of swimming activity was divided into three sessions, in each of which three sole were monitored simultaneously. A session lasted from 12 to 14 consecutive days: session I from 3 to 15 April; session II from 16 to 29 April; session III from 29 April to 13 May.

## Environmental variables

In order to assess the hydrological changes and bio-deposit accumulations from the oysters, we sampled the sediments and water in each zone. The locations of samples and probes are shown on Figure 1. Redox measurements were made on two series of 18 cores (80 mm diameter, 15 cm deep; 6 cores per O-, S- and F-zone), collected before and at the end of the experiment. The redox potential of each core was measured using the procedure given by Hussenot *et al.* (1994). Three measurements were made in the upper sediment layers (surface, -1.5 cm, and -3 cm), in all nine values per core. All measurements were made within 1 h of collection. Three sections of sediment 1-cm thick from each core were weighed, then oven-dried (60°C for about 72 h) to estimate the water content.

Six additional cores (i.e. 1/20<sup>th</sup> m<sup>2</sup> per zone) were collected at the beginning and the end of the experiment to determine the potential prey, i.e. the benthic fauna. After sieving to 0.5 mm, all organisms collected were stored in 70% alcohol until sorting, identification, and counting in the laboratory could be completed.

Hydrological data (temperature, oxygen, pH) were recorded at 10 min intervals during the course of the experiment by a central recording station (AOIP, Inc.). Temperature and oxygen probes (TriOxmatic 600 and pH meter 160, WTW Measurement Systems, Inc.) were fixed about 15 cm from the bottom. The pH probe was calibrated before installation in the pond using two buffered solutions (pH 7 and 4, Hanna Instruments, Hi 70007 and 70004). The pH measurements were made in the S-zone. Temperature and oxygen data were measured in the O- and F-zones only. An initial inter-calibration revealed that the average differences between probes were 0.12°C ( $\pm 0.07$ ) and 0.54% ( $\pm 2.37$ ) for temperature and oxygen, respectively. Further, the oxygen probe recordings were blocked at a maximum value of 120% for technical reasons, although *in situ* manual measurements occasionally gave values up to 200%. Data were nevertheless retained because our interest was in the variation of the measurements, and rather towards low than high values.

Every 3–5 days, water pond salinity was measured (portable thermo-salinometer WTW), and two water samples were collected from each zone, a total of 78 samples over the course of the experiment. After filtration with pre-combusted 1.2  $\mu\text{m}$  GF/F Whatman filters (450°C for 4 h), these samples were stored (-20°C) for analysis of dissolved nitrogen (complex nitrites/nitrates, N(NO<sub>2,3</sub>), ammoniac nitrogen N(NH<sub>3,4</sub>), and urea). The nitrogen analyses were done with a Carlo-Erba elemental analyser.

Two sediment traps (diameter 25 cm) were installed per zone on the pond bed, and deposits from the traps were collected every 3–5 days (78 samples in all). After decantation, excess water was pumped by hand and the deposited material dried in an oven (60°C for about 72 h), then weighed. Light penetration close to the bottom was also measured during daylight in each zone (quanta  $\text{cm}^{-2} \text{s}^{-1}$  measurements, given as % of subsurface light, quantummeter QSP 170, Biospherical, Inc.). We also used wind (direction and speed), irradiance ( $\text{W m}^{-2}$ ), rainfall ( $\text{mm m}^{-2}$ ), and atmospheric pressure (hPa) data from meteorological measurements routinely recorded in the CREMA field laboratory located in the same area as the mesocosm.

## Data analyses and statistics

Wind direction (in degrees) was converted to western-eastern (sine angle) and south-north (cosine angle) vector. The Pearson correlation coefficient method was used to evaluate relationships between environmental variables, with levels of significance calculated according to Scherrer's (1984) method. For these variables, between-session and/or zone comparisons were based on ANOVA, after assumptions made about normality and variance homogeneity rules were verified.

Sole swimming activity was evaluated using the hourly sum of distances travelled between consecutive position fixes (hourly distance,  $\text{m h}^{-1}$ ). As a first multifactor approach, we used a multilinear regression model (Scherrer, 1984) to evaluate the correlation between this variable and the meteorological and hydrological hourly averaged data, in order to identify link strength between variables. Daylight and night periods were identified, and twilight transition periods (1 h before sunrise and 1 h after sunset) were excluded from the data sets. Environmental variables were separated into classes, and their effects on swimming activity were assessed by comparing the hourly distances of all fish, regrouped into two data sets: day vs. night. We used non-parametric Kruskal-Wallis ANOVA (KW-ANOVA) to make comparisons, followed by non-parametric Dunn tests for multiple comparisons (Zar, 1984). Two-sample comparisons were assessed with a Mann-Whitney *U*-test. To quantify habitat preference, a frequentation rate was computed from the mean duration of residence per hour and per zone (in %). A temporal and spatial representation was chosen to present the evolution of sole distribution in the pond in relation to hour of the day. Statistics were created with SYSTAT 7.0, Excel 97, and XLSTAT 4.3 software packages.

## Results

### Mesocosm conditions: changes between sessions and zones

#### *Seasonal changes*

From session I to session III and independently of temporary fluctuations, water temperatures changes reflected spring warming (from 10°C to 24°C). Average values increased from  $12.5^\circ\text{C} \pm 1.6$  (session I) to  $17.0^\circ\text{C} \pm 2.0$  and  $16.5^\circ\text{C} \pm 1.6$  (sessions II and III; Figure 2a). The amplitude of daily temperatures ranged between 2 and 3°C. Average temperatures were similar between each zone for the same session, and observed differences were very close to the measurement errors on the probes. Salinity (not plotted) was also within the normal spring values for local ponds, from 31.0 to 32.5.

Dissolved oxygen varied from a low of 48% (minimum values registered in the morning and in the O-zone; Figure 2a) to >120%. Hourly average oxygen values were similar for all three sessions (from 100% to 105%), but there were daily fluctuations within each session, and night values were lower than in daylight. The daily fluctuations for oxygen ranged from

28% during session I (which increased after mid-April) to 36–37% (sessions II and III). For the duration of the experiment, oxygen averaged value for each session was significantly higher in the F- than in the O-zone (102–108% and 97–102%, respectively; ANOVA, d.f. = 5,  $F = 141.24$ ,  $p < 0.001$ ). There was more frequent and greater oversaturation by day in the F-zone, particularly during sessions II and III. Two events of low dissolved oxygen were recorded, <60% during the mornings of 11 and 12 May in the O-zone. Because the differences in dissolved oxygen between zones were relatively small, and values varied in accord, only dissolved O<sub>2</sub> values in the O-zone were used in the analysis of relationships between swimming activity and environmental variables.

Average pH variations were small, from pH 8.6 to 8.9 (hourly mean, Figure 2b), with a significant increase between session I and session III (from pH 8.7 to 8.9; ANOVA,  $F = 903.7$ ,  $p < 0.001$ , d.f. = 2). Daily ranges varied from a minimum of  $\pm 0.12$  pH units (session I) to a maximum of  $\pm 0.17$  pH units (session II). Fortnightly fluctuations decreased by 0.34 pH units during the second half of session II.

The most important water renewal took place during session II (16 pond filling/emptying events totalling 33.7 h), leading to variations in water column height that averaged 0.5 m, with a maximum of 1.7 m (Figure 2b). Smaller variations in water column height were recorded during sessions I and III. For example, during session III, water column height varied around 0.3 m, during 8 filling/emptying events that totalled 12.8 h. When the pond was filled to its minimum level (0.8 m), illumination was  $69 \pm 4\%$  of subsurface light, as measured near the bottom in both M- and F-zones. The illumination was reduced to  $4 \pm 2\%$  in S- and O-zones by shading from the trestles and oyster bags.

Atmospheric pressure variations correlated well with hydrological changes from cyclonic events (average  $1\,006\text{ hPa} \pm 5.7$  and  $1\,012\text{ hPa} \pm 4.6$  for sessions I and III, respectively; Figure 2c) and anticyclonic periods (session II average  $1\,021\text{ hPa} \pm 6.5$ ). Therefore, sessions differed as a result of rainfall and the associated wind. Rain fell more heavily during session I (7 days, totalling  $120\text{ mm m}^{-2}$ ), than during sessions III (6 days,  $50\text{ mm m}^{-2}$ ) and II (3 days,  $3\text{ mm m}^{-2}$ ). Hence, mean irradiance was significantly lower during session I ( $283 \pm 259\text{ W m}^{-2}$ ; Figure 2d) than during sessions II and III ( $444 \pm 304$  and  $390 \pm 317\text{ W m}^{-2}$ , respectively; ANOVA,  $F = 75.8$ ,  $p < 0.001$ , d.f. = 2). In terms of wind (Figure 2e), northeast winds prevailed during session I, and the highest speed was registered then ( $4.5 \pm 1.8\text{ m s}^{-1}$ ). More moderate westerly or changing wind regimes characterized sessions II and III ( $3.6 \pm 2.0\text{ m s}^{-1}$  and  $4.4 \pm 1.7\text{ m s}^{-1}$ , respectively). There were two high wind events (mean speed  $>5\text{ m s}^{-1}$ , and gales  $>8\text{ m s}^{-1}$ ) during session I (7–15 April, northeast winds) and also between sessions II and III (from 26 April to 3 May, southwest winds).

Relationships between the hydrological and climatic data were significant: a positive correlation between atmospheric pressure and pond temperature, oxygen, and pH, and a negative correlation between water-column height and pH (Table 2). Water-renewal rates were not measured directly, but the pH measurements indirectly showed the effects of water renewal in the mesocosm on the physico-chemical characteristics of the pond, and were therefore a good indicator of water-renewal events.

#### *Oyster-induced changes*

Sedimentation rates increased significantly from session I to sessions II and III. At the same time, the only change in nitrogen was an appreciable decrease in nitrate, as inferred from the variations in the nitrite/nitrate complex ( $\text{N}(\text{NO}_{2,3})$ ; Table 3a). In contrast, the O-zone evolved significantly differently from the other two zones (Table 3b). This difference concerned most of the nitrogen compounds (nitrites, urea, and ammonia nitrogen) and sedimentation rates (3–5 times higher in the O-zone). Despite the absence of significant differences between the redox potential of bottom sediments from the three zones (Table 4), there was a significant

change in the redox potential between the initial and final measurements. Negative redox potentials at the end of the experiment showed that sediments were more deoxygenated in all zones. A water-content increase in the upper sediment layer (0–3 cm) also suggested significant change, but was limited to O-zone sediments.

The invertebrates recovered from core samples were mainly small in size, undetermined meiofaunal species (foraminiferans, ostracods, harpacticoid copepods, and oligochaetes) and a few macrofaunal species (Figure 3; mostly the annelids, *Hedistes diversicolor* and *Streblospio* sp., and bivalves, *Abra* spp.). There was no obvious difference over time in any zone, except for an increase in the occurrence of harpacticoid copepods and *Hedistes* juveniles.

### Sole swimming activity

#### *Diel activity*

Except for sole 2 (session I), all fish had a pronounced daily cycle (Figure 4). Individual sole average activity levels were significantly different by day, and less (16.0–36.6 m h<sup>-1</sup>) than at night (21.7–45.7 m h<sup>-1</sup>). In addition, differences in activity levels between sole were significant. This allowed us to test for between-session differences or similarities, among different responses and according to day or night activity (Table 5). Session II had the highest activity levels of sole 4, 5, and 6 (groups III–V), by both night and day, whereas by day in sessions I and III, activity was least (group I). At night the least active sole were from session I, except for two sole (numbers 7 and 9) from session III.

#### *Habitat selection*

Fish moved along the enclosure border but also occupied each zone differently depending on both individual and day-night cycle (see Zone diagrams in Figure 5). Given three plus one habitat choices, the oyster zone (O) was the main location for daytime resting: 6 of 9 sole stayed there, and frequentation rates ranged from 78% to 98% (sole numbers 2, 3, 4, 5, 6, and 9). Only three sole selected different habitat: sole 1 and 7 used mainly the S-zone (52% and 89%, respectively), and sole 8 was almost exclusively in the F-zone (90%). By night, all sole travelled through most of the enclosed area and visited zones where they stayed for only a short time or not at all by day. For example, sole numbers 2, 3, 4, 5, 6, and 9 intensified their F- and/or S-zone frequentation rates at night (Figure 5). However, the O-zone remained the preferred habitat for sole 2, 3, 6, and 9, followed by sole 4 and 5, and it was the zone least frequently selected by sole 1 and 7.

Wind-regime changes interfered with the day-night cycle of exploratory activity and habitat choice. For example, sole 1, whose main choice was the S-zone during calm weather (Figure 6a), moved to the O-zone during east turning northeast wind (>5 m s<sup>-1</sup>) days (Figure 6b). In another example, sole 4, which was both the most active and diel fish, alternated between daylight O-zone occupancy and nocturnal frequentation of other zones (Figure 6c). This rotation of habitats stopped under windy conditions on 28 April (Figure 6d, southwest winds >5 m s<sup>-1</sup>). Sole 8 differed from the others, not only from the two other session III occupants, but also from the six sole in the other two sessions. Sole 8 was among the least active by day and, although it had an average night-time activity level, its movements were restricted to the F-zone, entering the S-zone only occasionally (Figure 6e).

#### *Swimming activity and environmental variations*

Multilinear regression analysis showed that swimming activity and environmental data were significantly linked: positively with oxygen content (coefficient 0.25) and atmospheric pressure (0.58), and negatively with light level (–0.01), water-column height in the pond (–0.90), and pH (–23.87;  $n = 2322$ , d.f. = 5,  $r = 0.40$ ).

The combination of factors used in the multilinear model explained only 40% of the variability in swimming activity. Therefore, univariate analyses were used to further compare other variable classes (temperature and wind data). They revealed a temperature effect that depended mainly on enhanced swimming activity under the highest temperature by day (18, 20, and 21°C; Figure 7a). At night, swimming activity peaked when temperatures were >15°C, with hourly distances covered averaging between 30 and 36 m h<sup>-1</sup>. In the 10–14°C temperature range, hourly distances decreased significantly, to 20–26 m h<sup>-1</sup> (KW-ANOVA;  $H = 66$ ,  $p < 0.001$ , and  $H = 78$ ,  $p < 0.001$ ). In terms of wind effects (Figure 7b), and if there was no difference in swimming activity attributable to wind speed by day (KW-ANOVA;  $H = 11.4$ , d.f. = 9,  $n = 1\,487$ ,  $p = 0.25$ ), significant differences appeared at night (KW-ANOVA;  $H = 30.1$ , d.f. = 9,  $n = 1\,068$ ,  $p < 0.001$ ). This analysis allowed us to infer trends between low rates of swimming activity observed for the 5 m s<sup>-1</sup> wind class and rates for the 2 and 3 m s<sup>-1</sup> classes. Moreover, significant differences in swimming activity existed depending on wind direction (KW-ANOVA for day and night periods, respectively,  $H = 32.5$  and 65.9, d.f. = 10,  $n = 1\,487$   $p < 0.001$  for north-south sectors;  $H = 33.4$  and 65.0, d.f. = 10,  $n = 1\,487$ ,  $p < 0.001$  for east-west sectors). By day, mainly the westerly wind sector and, to a lesser extent, the southerly sector, caused an increase in hourly distances covered by fish (Figure 7c, d). By night, the distances travelled hourly decreased for both the north and east sectors, compared with the south and north sectors.

## Discussion

In this study we recreated habitat conditions in a mesocosm that include shellfish culture installations similar to those nearby in the Bay of Biscay. Such installations reduce the free surface area, artificially structure the habitat, and alter the water and sediment characteristics. The experimental framework of this study necessarily imposed some constraints that need to be aired before provisional conclusions regarding the effects of shellfish farming on sole swimming behaviour can be made.

### Field habitat vs. mesocosm

The experiment restructured the saltmarsh pond habitat using a rigid enclosure, and dividing the marsh into three zones orientated differently to the wind. Therefore, depending on the wind regime, different zones were exposed to wind-generated ambient noise and amplified by the enclosure. Lagardère *et al.* (1994) showed that sole prefer to stay in a sheltered area of an enclosure where ambient noise levels are lowest when winds gust to >8 m s<sup>-1</sup>. In our experiment, the observed responses differed because sole moved towards the wind-exposed area of the enclosure. This difference could be due to the lower intensity of the wind gusts and/or to the PVC netting used for the enclosure, which possibly vibrated and may have increased wind-generated noise close to the net and modified wind-orientated fish response.

The saltmarsh site used for the mesocosm is a semi-closed shallow pond where the environmental variability of a natural nursery is enhanced for all hydrological and climatic variables, including light. As a former oyster pond, it functions as a sedimentation basin (Héral, 1989), and it contains less turbid water than the adjacent open waters of the sole nurseries. Indeed, light penetration to the bottom of the pond was measured at about 70% (0.6 m depth in the F-zone). By comparison, intertidal mudflats are described as having very little light penetration when immersed (Guarini *et al.*, 2000). In subtidal areas, a similar situation potentially exists when tidal or wind-induced currents are sufficiently strong. For example, during a summer 2002 cruise in the coastal waters of the Charentais Straits, light

penetration was zero near the seabed, between 6 and 10 m deep depending on location (PL, unpublished data).

The pond management plan, in which water is renewed by tidal exchange, was initiated in January, ensuring colonization by invertebrate fauna particular to the system (Reymond, 1991). However, water entry was irregular and infrequent, and no fertilization to stimulate primary benthic production was made (Hussenot, 2001) to preclude additional perturbation of the system other than by the oyster-trestle installations. For the duration of the spring experiment, macrofauna quantity did not increase. Therefore, the initial fish stocking density was rather high ( $15 \text{ g m}^{-2}$ ) compared with a recommended stocking density of  $5\text{--}10 \text{ g m}^{-2}$  for on-growing of fish in extensive pond aquaculture (J. Hussenot, pers. comm.).

Previous studies using telemetry have shown that sole recover normal swimming behaviour three days after external tagging (Lagardère *et al.*, 1988). Because the first position recordings started five days after tagging and transfer to the pond, we assumed that the fish were accustomed to their new environment and had recovered from capture and tagging stress. A parallel study revealed medium-term effects for tagged sole, including a slight mass loss, whereas prey quantity seemed sufficient to allow the control fish in the same mesocosm and over the same period to grow significantly (Bégout Anras *et al.*, 2003). Therefore, even if we could not perform individual feed intake monitoring under our experimental set-up, we are confident that our sole had sufficient food to eat should they desire it. However, we do acknowledge that the tagged sole here compared in terms of swimming behaviour had similar weight loss as in the other reported experiment, a loss attributable to tagging. These effects of external tagging on sole appeared for a tag weight to fish wet weight ratio representing between 2.8 and 3.6%. This could perhaps be explained by drag forces linked to the additional burden caused by the tag, which creates extra energy needs for a similar swimming efficiency (+5–15%, depending on fish size; Arnold and Holford, 1978).

Our telemetry system allowed many tag frequencies to be sampled in sequence, and we chose to track three fish per fortnightly session during the 45-day experiment. We recorded one location per minute per sole, which caused some loss of information on swimming trajectory and decreased the estimates of swimming amplitude (Lagardère *et al.*, 1990; Løkkeborg *et al.*, 2002). Therefore, activity levels reported here, an average night activity of  $30 \text{ m h}^{-1}$  and a maximum of  $178 \text{ m h}^{-1}$  for water temperatures above  $10^\circ\text{C}$ , would be on the low side. These data can be compared between fish and sessions because the sampling rate was constant for all three sessions, but they are not comparable with other documented values where the sampling rate was greater. For example, swimming activities between  $72\text{--}720 \text{ m h}^{-1}$  have been measured in a similar marsh enclosure for temperatures above  $8^\circ\text{C}$  using a location sampling rate of 10–15 s (Lagardère and Sureau, 1989; Lagardère *et al.*, 1994).

### Swimming activity

In the mesocosm, eight sole out of nine had a diel cycle with marked nocturnal activity that is typical of both juvenile and adult sole in the natural environment (Kruuk, 1963; Greer Walker *et al.*, 1980; Lagardère, 1987). Light avoidance begins with metamorphosis (Blaxter and Staines, 1970), and sole develop sensitivity to low light thresholds under conditions of high light level (Blaxter, 1986; Champalbert *et al.*, 1991). These particularities of sole suggest that a shallow marsh habitat, which has greater light penetration, may influence daylight responses and reinforce burying behaviour (Ellis *et al.*, 1997). Depending on weather conditions, changes in daylight swimming activity were observed from session I (mostly overcast weather) to III; this was particularly true for sole 8, located in the F-zone during session III.

Swimming amplitude levels varied significantly between sessions, and session II had the highest values (by day and night); that session coincided with a warm phase linked to

fluctuations in seasonal hydrological and climatic factors at the experimental site. Temperature controls a fish's physiological processes and determines behavioural adjustments (Neill *et al.*, 1994; Gibson, 1997; Morgan and Metcalfe, 2001), but the observed nocturnal increase in swimming activity for temperatures above 15°C can only partially be explained by temperature. Water temperature variations measured during the experiment were low; sole is an eurythermic organism capable of maintaining a metabolic capacity of >80% for a temperature range of 13.3–22.9°C (Lefrançois and Claireaux, 2003). Furthermore, a temperature decrease observed during session I (minimum of 10°C) was the only potential limiting factor, but this remained well above the threshold value of 5°C known to reduce sole swimming activity significantly ( $\leq 5^\circ\text{C}$ ; Lagardère and Sureau, 1989).

Under the experimental conditions, factors other than temperature, including variables linked to pond management and confinement time, altered the pond's environment and possibly influenced swimming activity. For example, oxygen and pH fluctuated daily, with lower values at night owing to the activity of organisms present in the pond (mainly micro- and macroalgae), and dissolved oxygen is known to act directly on biological processes. Oxygen super-saturation episodes were frequent during daylight, but no significant effects on metabolic rate or heart rate have been documented in experimental studies (Lefrançois and Claireaux, 2003). For low oxygen concentration, the lower limit for active sole metabolism is between 40 and 60% oxygen saturation (Van den Thillart *et al.*, 1994). Such low values were occasionally measured in the early morning during our experiment.

Relationships with other environmental variables were more difficult to establish. They could have direct (i.e. irradiance) or indirect (i.e. tidal cycle, atmospheric pressure) effects and possibly obscure the influence of other factors. As an example, we observed increased sole swimming activity during session II coinciding with greatest water renewal (spring tide and full moon). The water movement induces hydrostatic pressure changes that represent one of the main synchronizers of the tidal cycle for fish (Gibson, 2003). Tidal cycles could be perceived indirectly in the mesocosm through water renewal and water height changes. Atmospheric pressure appeared as a major explanatory factor of activity changes: activity tended to increase with increasing anticyclonic tendency. As an integrative factor, atmospheric pressure was positively linked to temperature, and also to oxygen and pH. Considering either shallow marshes or coastal zones, one could question the extent to which changes in atmospheric pressure are perceived either through associated hydrological changes, or directly, as an early indicator of weather changes. The latter has been suggested for seabass (*Dicentrarchus labrax*) in similar mesocosms (Bégout Anras and Lagardère, 1998), and has been supported more recently by a study on young shark *Carcharhinus limbatus* before a tropical twister (Heupel *et al.*, 2003).

Weather conditions were significantly different between sessions, and also varied within the fortnight period of each session. Exposed to the same environmental variations, sole had significant inter-individual differences in behavioural response: each adjusted its own endogenous rhythm, particularly during session II, when the overall temperature increase favoured swimming. During session III, a different situation arose with swimming levels similar to session I, despite higher temperatures that stabilized at around 15°C. The slightly altered status observed for most sole and attributed to tagging (discussed above), may have contributed to the general levelling off of swimming activity during session III. Stress may also be responsible for small or severe activity changes in fish, perhaps for reasons of social interaction (Gomez-Laplaza and Morgan, 2003) or through suffering experimental confinement (Sneddon, 2003). In the present study, changes in the amplitude of the daily swimming rhythm were observed, but the rhythms remained consistent. The observed diversity in individual responses therefore suggest that constraints imposed by the

experimental conditions did not reach a critical threshold capable of modifying behavioural response (Schreck *et al.*, 1997; Kristiansen *et al.*, 2004).

#### Utilization of habitat under shellfish culture installations

In addition to the hydrological and climatic changes that orientated and modified activity levels, spatial orientation and structure, and perennial quality of the habitat are key determinants. We attempted to minimize perturbations other than those created by the shellfish-culture trestles and the live oysters in the mesocosm. Even if the enclosure limits acted temporarily on swimming activity, trestle structures, with bags of live oysters or shells, were the most important elements governing the spatial distribution of sole.

Given the same water quality, mechanisms of habitat selection by flatfish can be divided into biological and sedimentary characteristics (cues) and indicators that allow a fish to locate the habitat (clues), along with a learning phase (Gibson, 1997). For sole, a nocturnal benthic species, preferred habitats are mostly soft substrata because they allow the fish to bury themselves by day. These types of sediments may contain mud and organic matter preferred by suitable prey, such as annelids (Salen-Picard *et al.*, 2002). Eight sole out of nine sheltered under the cover of oyster trestles by day, but we could not determine with our monitoring system whether or not sole were actually buried. Nevertheless, we noted slight daytime activity in both zones O and S, compared with the daytime immobility of the one sole that remained in the F-zone. Comparing results between the three sessions suggested that factors other than light were important in the selection of habitat zones O and S. We hypothesize that oyster trestles offered cover, camouflage, and safety and were therefore attractive to sole (as artificial reef-structuring effects).

In shellfish ponds, oysters enhance the vertical flux of matter through their filtration activity and the production of faeces/pseudofaeces (Sornin, 1981). Enrichment in nitrogen (ammonia in particular) is one major modification of oyster production (Feuillet-Girard *et al.*, 1988; Dame *et al.*, 1989). Oysters also reduce the depth into the sediment of the oxygenated layer and increase the organic matter content (Nugues *et al.*, 1996). Although our experiment was limited in space and time, similar habitat modifications were observed: bio-deposits by oysters in O-zone significantly modified the fine sediment covering the pond bed and water quality. Increased sedimentation fluxes under live oysters were 3–5 times greater than those measured in zones S and F, and were similar to levels measured along the coast (Feuillet-Girard *et al.*, 1994; Nugues *et al.*, 1996). The same conclusion could be reached for the increase in dissolved nitrate (Feuillet-Girard *et al.*, 1988). However, nitrate components, known potentially to alter fish behaviour, remained at levels 100 or 1000 times below toxic levels (Bianchini *et al.*, 1996; Dosdat *et al.*, 2003; Foss *et al.*, 2003).

Nocturnal movements out of the O-zone may correspond to both exploratory behaviour and temporary escape from a less favourable habitat with a night-time low level of oxygen. Site fidelity was also observed, with fish returning (even under low morning oxygen levels) to the same sheltered spot they occupied the previous day. For example, sole 4 and 8 came back to almost identical sites in zones O and F, respectively, after active nocturnal swimming, sometimes associated with climatic events. This exploration of all zones of the enclosure could be linked to foraging. Furthermore, significant modifications of benthic fauna have been observed in oyster-culture areas (Castel *et al.*, 1989; Nugues *et al.*, 1996), with increasing numbers of species that are less sensitive to organic matter enrichment, and in particular, meiofauna overtaking macrofauna in abundance. Our experiment was probably too short to accommodate such benthic fauna enrichment. The one exception was for the harpacticoid copepods that began to appear in every zone. If most of the available macrofauna (*Hedistes diversicolor*, *Abra* spp.) was consumed, towards the end of the experiment only

juveniles could replace it. Sole, however, are opportunistic predators (Cabral, 2000; Sorbe, 1981), capable of eating fishpond insect fauna (Lasserre and Lasserre, 1978), which may explain the relative insensitivity of their behaviour here to available prey type.

## Conclusions

Sole swimming behaviour in a pond mesocosm was largely driven by day-night cycles: amplitude changes were associated with hydrological and climatic variables, for which individual effects could not be discerned. The presence of oyster-culture trestles did not significantly modify patterns of sole swimming. Under the experimental conditions, hydrological and bio-sedimentary changes linked to oyster-trestle culture did not reduce access to the habitat under the structure. In contrast, the culture system created microhabitat more frequented by fish than the homogeneous open area.

Complementary field studies will be necessary before we can extrapolate our results to coastal nurseries where shellfish culture is also practiced, although the potential behavioural responses observed here could serve as reference for future field studies. The present study illustrated the adaptive capacity of sole to habitat modification by oyster culture. Sole, within the microhabitats offered in the mesocosm, displayed some capabilities for habitat recognition. Recently acknowledged for inferior vertebrates such as fish, these cognitive capabilities permit greater flexibility and adaptability (see a review in Odling-Smee and Braithwaite, 2003). Taking into account such capabilities will be essential for better understanding patterns of movement and habitat selection in the wild.

## Acknowledgements

We thank G. Dupont for his valuable field help, J. M. Guarini for translation and initial review, and an anonymous reviewer at submission. This research programme was supported by IFREMER Défi “Golfe de Gascogne”, the National Programme on Coastal Environment (PNEC), and a PhD grant to PL from the Conseil Général de la Charente Maritime. The study was conducted under the approval of the Animal Care Committee of France under the official licence of J. P. Lagardère (n° 02526).

## References

- Amara, R., Lagardère, F., Désaunay, Y., and Marchand, J. 2000. Metamorphosis and estuarine colonisation in the common sole, *Solea solea* (L.): implications for recruitment regulation. *Oceanologica Acta*, 23: 469–484.
- Arnold, G. P., and Holford, B. H. 1978. The physical effects of an acoustic tag on the swimming performance of plaice and cod. *Journal du Conseil International pour l'Exploration de la Mer*, 38: 189–200.
- Beck, M. W., Heck, K. L., Able, K. W., Childers, D. L., Eggleston, D. B., Gillanders, B. M., Halpern, B., Hays, C. G., Hoshino, K., Minello, T. J., Orth, R. J., Sheridan, P. F., and Weinstein, M. P. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience*, 51: 633–641.
- Bégout Anras, M. L., and Lagardère, J. P. 1998. Weather related variability. Consequences on the swimming activity of a marine fish. *Comptes Rendus de l'Academie des Sciences – Series III – Sciences de la Vie*, 321: 641–648.
- Bégout Anras, M. L., Covès, D., Dutto, G., Laffargue, P., and Lagardère, F. 2003. Tagging juvenile seabass and sole with telemetry transmitters: medium-term effects on growth. *ICES Journal of Marine Science*, 60: 1328–1334.
- Bianchini, A., Wasielesky, W., and Filho, K. C. M. 1996. Toxicity of nitrogenous compounds to juveniles of flatfish *Paralichthys orbignyanus*. *Bulletin of Environmental Contamination and Toxicology*, 56: 453–459.
- Blaxter, J. H. S. 1986. Development of sense organs and behaviour of teleost larvae with special reference to feeding and predator avoidance. *Transactions of the American Fisheries Society*, 115: 98–114.
- Blaxter, J. H. S., and Staines, M. 1970. Pure-cone retinæ and retinomotor responses in larval teleosts. *Journal of the Marine Biological Association of the UK*, 50: 449–460.
- Cabral, H. N. 2000. Comparative feeding ecology of sympatric *Solea solea* and *S. senegalensis*, within the nursery areas of the Tagus Estuary, Portugal. *Journal of Fish Biology*, 57: 1550–1562.
- Castel, J., Labourg, P.-J., Auby, I., Escavaraige, V., and Garcia, M. E. 1989. Influence of seagrass beds and oyster parks on the abundance and biomass patterns of meio- and macrobenthos in tidal flats. *Estuarine, Coastal and Shelf Science*, 28: 71–85.
- Champalbert, G., Macquart-Moulin, C., Patrìti, G., and Chiki, D. 1991. Ontogenic variations in the phototaxis of larval and juvenile sole *Solea solea* L. *Journal of Experimental Marine Biology and Ecology*, 149: 207–225.
- Cote, D., Ollerhead, L. M. N., Scruton, D. A., and McKinley, R. S. 2003. Microhabitat use of juvenile Atlantic cod in a coastal area of Newfoundland determined by 2D telemetry. *Marine Ecology Progress Series*, 265: 227–234.
- Dame, R. F., Spurrier, J. D., and Wolaver, T. G. 1989. Carbon, nitrogen and phosphorus processing by an oyster reef. *Marine Ecology Progress Series*, 54: 249–256.
- Daskalov, G. M. 2003. Long-term changes in fish abundance and environmental indices in the Black Sea. *Marine Ecology Progress Series*, 255: 259–270.
- Dosdat, A., Person-Le Ruyet, J., Coves, D., Dutto, G., Gasset, E., Le Roux, A., and Lemarie, G. 2003. Effect of chronic exposure to ammonia on growth, food utilisation and metabolism of the European sea bass (*Dicentrarchus labrax*). *Aquatic Living Resources*, 16: 509–520.
- Ellis, T., Howell, B. R., and Hughes, R. N. 1997. The cryptic responses of hatchery-reared sole to a natural sand substratum. *Journal of Fish Biology*, 51: 389–401.

- Feuillet-Girard, M., Héral, M., Abrioux, M. F., and Fontugne, M. 1994. Carbone organique dissous et particulaire de la colonne d'eau et de l'interface eau-sédiment du bassin de Marennes-Oléron: influence des huîtres. *Oceanologica Acta*, 17: 271–284.
- Feuillet-Girard, M., Héral, M., Sornin, J-M., Deslous-Paoli, J. M., Robert, J. M., Mornet, F., and Razet, D. 1988. Eléments azotés de la colonne d'eau et de l'interface eau-sédiment du bassin de Marennes-Oléron : influence des cultures d'huîtres. *Aquatic Living Resources*, 1: 251–265.
- Foss, A., Evensen, T. H., Vollen, T., and Oiestad, V. 2003. Effects of chronic ammonia exposure on growth and food conversion efficiency in juvenile spotted wolffish. *Aquaculture*, 228: 215–224.
- Gibson, R. N. 1997. Behaviour and the distribution of flatfishes. *Journal of Sea Research*, 37: 241–256.
- Gibson, R. N. 2003. Go with the flow: tidal migration in marine animals. *Hydrobiologia*, 503: 153–161.
- Gomez-Laplaza, L. M., and Morgan, E. 2003. The influence of social rank in the angelfish, *Pterophyllum scalare*, on locomotor and feeding activities in a novel environment. *Laboratory Animals*, 37: 108–120.
- Greer Walker, M., Riley, J. D., and Emerson, L. 1980. On the movements of sole (*Solea solea*) and dogfish (*Scyliorhinus canicula*) tracked off the East Anglia coast. *Netherlands Journal of Sea Research*, 14: 66–77.
- Guarini, J. M., Blanchard, G. F., Gros, P., Gouleau, D., and Bacher, C. 2000. Dynamic model of the short-term variability of microphytobenthic biomass on temperate intertidal mudflats. *Marine Ecology Progress Series*, 195: 291–303.
- Héral, M. 1989. L'ostréiculture française traditionnelle oyster culture in France. *In Aquaculture*, 1, 2<sup>nd</sup> edn, pp. 348–397. Ed. by G. Barnabé. Techniques et Documentations Lavoisier, Paris.
- Heupel, M. R., Simpfendorfer, C. A., and Hueter, R. E. 2003. Running before the storm: blacktip sharks respond to falling barometric pressure associated with Tropical Storm Gabrielle. *Journal of Fish Biology*, 63: 1357–1363.
- Huntingford, F. A. 1993. Can cost-benefit analysis explain fish distribution patterns? *Journal of Fish Biology*, 43(Suppl. A): 289–308.
- Hussenot, J. 2001. Comment une approche écologique peut aider au développement de nouvelles filières d'élevage dans les marais salés endigués? *Journal de Recherche Océanographique*, 26: 110–112.
- Hussenot, J., Martin, J. L., Feuillet-Girard, M., and Gautier, D. 1994. Evaluation de la qualité des sédiments en aquaculture par des techniques simples et rapides. *In Measures for Success*. Ed. by P. Williot, European Aquaculture Society Special Publication 21: 47-48..
- Klimley, A. P., Le Boeuf, B. J., Cantara, K. M., Richert, J. E., Davis, S. F., and Van Sommeran, S. 2001. Radio-acoustic positioning as a tool for studying site-specific behavior of the white shark and other large marine species. *Marine Biology*, 138: 429–446.
- Kristiansen, T. S., Fernö, A., Holm, J. C., Privitera, L., Bakke, S., and Fosseidengen, J.E. 2004. Swimming behaviour as an indicator of low growth rate and impaired welfare in Atlantic halibut (*Hippoglossus hippoglossus* L.) reared at three stocking densities. *Aquaculture*, 230: 137–151.
- Kruuk, H. 1963. Diurnal periodicity in the activity of the common sole, *Solea vulgaris* Quensel. *Netherlands Journal of Sea Research*, 2: 1–28.
- Lagardère, J. P. 1987. Feeding ecology and daily food consumption of common sole, *Solea vulgaris* Quensel, juveniles on the French Atlantic coast. *Journal of Fish Biology*, 30: 91–104.

- Lagardère, J. P., Bégout, M-L., Lafaye, J-Y., and Villotte, J. P. 1994. Influence of wind-produced noise on orientation in the sole (*Solea solea*). *Canadian Journal of Fisheries and Aquatic Sciences*, 51: 1258–1264.
- Lagardère, J-P., Ducamp, J. J., Favre, L., Dupin, J. M., and Sperandio, M. 1990. A method for the quantitative evaluation of fish movements in salt ponds by acoustic telemetry. *Journal of Experimental Marine Biology and Ecology*, 141: 221–236.
- Lagardère, J. P., Ducamp, J. J., Frikha, L., and Sperandio, M. 1988. Ultrasonic tracking of common sole juveniles (*Solea vulgaris* Quensel, 1806) in a saltmarsh: methods and fish response to some environmental factors. *Journal of Applied Ichthyology*, 4: 87–96.
- Lagardère, J. P., and Sureau, D. 1989. Changes in the swimming activity of the sole (*Solea vulgaris* Quensel, 1806) in relation to winter temperatures in a saltmarsh: observations using ultrasonic telemetry. *Fisheries Research*, 7: 233–239.
- Lasserre, G., and Lasserre, P. 1978. Croissance, production et demande d'énergie d'une population de soles (*Solea vulgaris*) introduite dans une lagune aménagée (Bassin d'Arcachon). *Publications Scientifiques et Techniques du Centre National pour l'Exploitation des Océans (CNEXO), Série: Actes de Colloques*, 7: 689–712.
- Lefrançois, C., and Claireaux, G. 2003. Influence of ambient oxygenation and temperature on metabolic scope and heart rate of the common sole (*Solea solea*). *Marine Ecology Progress Series*, 259: 273–284.
- Lê, K. L. 1983. Les phases initiales du cycle biologique (oeufs, larves et jeunes) de *Solea vulgaris* Quensel et *Solea senegalensis* Kaup (Poissons, Pleuronectiformes, Soleidae); relations avec le bassin de Marennes-Oléron, écosystème estuarien à vocation aquicole. PhD thesis, University of Aix-Marseille II, France. 136 pp.
- Le Pape, O., Chauvet, F., Mahevas, S., Lazure, P., Guérault, D., and Désaunay, Y. 2003. Quantitative description of habitat suitability for the juvenile common sole (*Solea solea*, L.) in the Bay of Biscay (France) and the contribution of different habitats to the adult population. *Journal of Sea Research*, 50: 139–149.
- Løkkeborg, S., Fernö, A., and Jørgensen, T. 2002. Effect of position-fixing interval on estimated swimming speed and movement pattern of fish tracked with a stationary positioning system. *Hydrobiologia*, 483: 259–264.
- Marchand, J., and Masson, G. 1989. Process of estuarine colonisation by 0-group sole (*Solea solea*): hydrological conditions, behaviour, and feeding activity in the Vilaine estuary. *Rapports et Procès-Verbaux des Réunions de Conseil International pour l'Exploration de la Mer*, 191: 287–295.
- Morgan, I. J., and Metcalfe, N. B. 2001. The influence of energetic requirements on the preferred temperature of overwintering juvenile Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences*, 58: 762–768.
- Neill, W. H., Miller, J. M., Van der Veer, H. W., and Winemiller, K. O. 1994. Ecophysiology of marine fish recruitment: a conceptual framework for understanding interannual variability. *Netherlands Journal of Sea Research*, 32: 135–152.
- Nugues, M. M., Kaiser, M. J., Spencer, B. E., and Edwards, D. B. 1996. Benthic community changes associated with intertidal oyster cultivation. *Aquaculture Research*, 27: 913–924.
- Odling-Smee, L., and Braithwaite, V. A., 2003. The role of learning in fish orientation. *Fish and Fisheries*, 4: 235–246.
- Reymond, H. 1991. Dynamique de la chaîne hétérotrophe benthique des marais maritimes en période estivale et son impact sur les productions aquacoles de carnivores: *Penaeus japonicus*, un modèle d'étude. Thèse de Université de Paris 6. 257 pp.
- Salen-Picard, C., Darnaude, A., Arlhac, D., Harmelin-Vivien, M. L. 2002. Fluctuations of macrobenthic populations: a link between climate-driven river run-off and sole fishery yields in the Gulf of Lions. *Oecologia*, 133: 380–388.

- Scherrer, B. 1984. Biostatistique. Gaëtan Morin, Montréal. 850 pp.
- Schreck, C. B., Olla, B. L., and Davis, M. W. 1997. Behavioral responses to stress. *In* Fish Stress and Health in Aquaculture, pp. 145–170. Ed by G. K. Iwama, A. D. Pickering, J. P. Sumpter, and C. B. Schreck. Cambridge University Press, Cambridge, UK.
- Sneddon, L. U. 2003. The evidence for pain in fish: the use of morphine as an analgesic. *Applied Animal Behaviour Science*, 83: 153–162.
- Sorbe, J. C. 1981. Rôle du benthos dans le régime alimentaire des poissons démersaux du secteur Sud Gascogne. *Kieler Meeresforschungen Sonderheft*, 5: 479–489.
- Sornin, J-M. 1981. Processus sédimentaires et biodéposition liés à différents modes de conchyliculture – Baie de Cancale, Anse de l'Aiguillon et Bassin de Marennes Oléron. PhD thesis, University of Nantes, France.
- Van den Thillart, G., Dalla Via, J., Vitali, G., and Cortesi, P. 1994. Influence of long-term hypoxia exposure on the energy metabolism of *Solea solea*. 1. Critical O<sub>2</sub> levels for aerobic and anaerobic metabolism. *Marine Ecology Progress Series*, 104: 109–117.
- Van der Veer, H. W., Dapper, R., and Witte, J. I. J. 2001. The nursery function on the intertidal areas in the western Wadden Sea for 0-group sole *Solea solea* (L.). *Journal of Sea Research*, 45: 271–279.
- Zar, J. H. 1984. *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, New Jersey. 718 pp.

Figure 1. Diagram of the experimental set-up showing pond borders (continuous line) and enclosure outlines (stippled line, the white rectangle indicates a gate), delimiting three different zones: O, live oysters on trestles (grey rectangles represent oyster bags); S, oyster shells only in bags also on trestles; F, free zone; M, the intermediate (junction) zone. Hydrological data were taken at different locations: crosses indicate temperature and oxygen probes in the O- and F-zones, and a pH probe in the S-zone only; filled squares indicate sediment traps and water samples. Outside the enclosure, grey triangles indicate the hydrophone positions for acoustic telemetry, and the grey circle shows the location of the pipe regulating water filling (pipe height = 0.8 m).

Figure 1

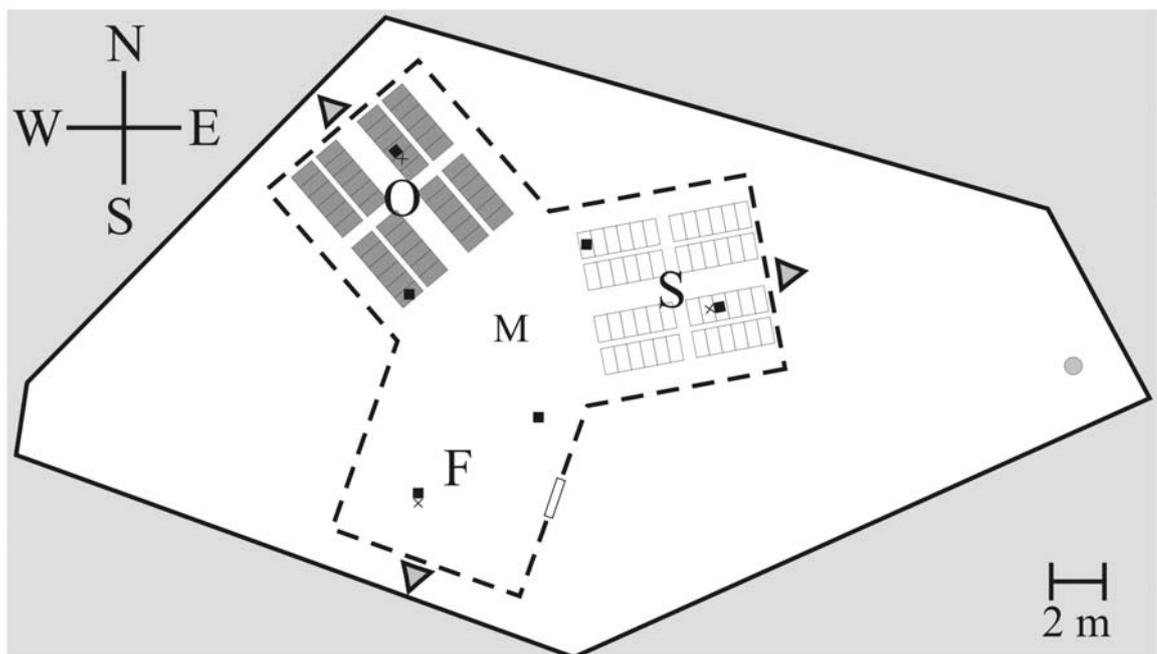


Figure 2. Time-series of environmental factors recorded during the 3 experimental sessions (I, II and III). (a) Temperature (continuous line) and oxygen (dotted line, oxygen probe records blocked at a maximum of 120% for technical reasons); (b) pH (continuous line), water column height (dotted line) and moon phases (full and new moons, white and black discs); (c) atmospheric pressure (continuous line) and rainfall (vertical bars); (d) solar irradiance; (e) wind speed and direction hodographs.

Figure 2

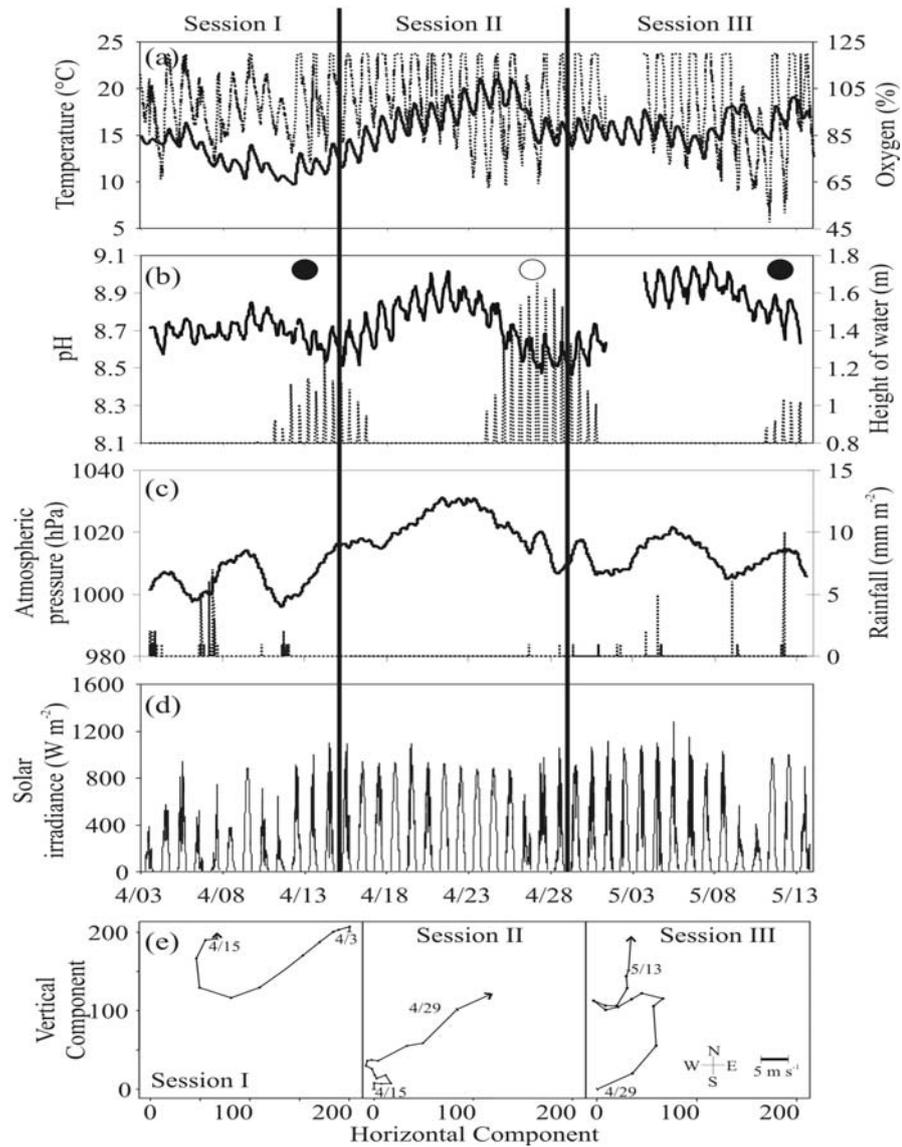


Figure 3. Mean numbers ( $\pm$  s.d.) of core-sampled invertebrates in zones O, S, and F at the beginning (white bars) and end of the experiment (black bars). (pl: planktonic copepods, harp: harpacticoids).

Figure 3

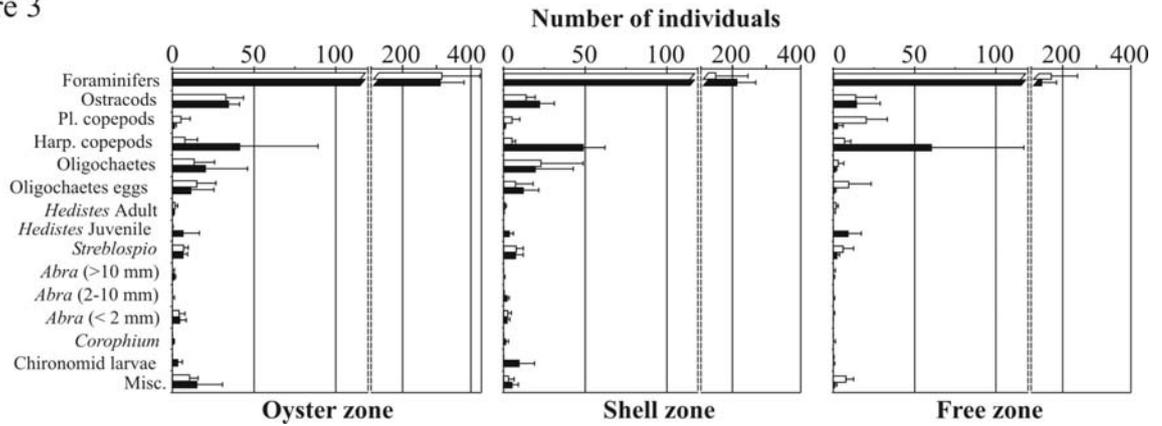


Figure 4. Sole average hourly swimming activity (and 95% confidence interval) by day (white bars) and night (black bars). NS signifies a non-significant difference between day and night (Mann Whitney  $U$ -test = 8.58, d.f. = 1,  $n$  = 261,  $p$  = 0.6).

Figure 4

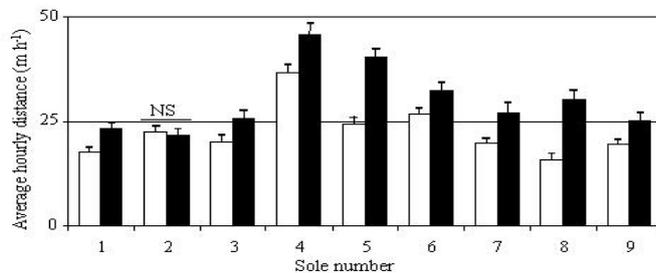


Figure 5. Sole swimming activity and habitat selection of three simultaneously tracked sole during each session. Upper graphs give mean hourly distances ( $\text{m h}^{-1} \pm \text{s.d.}$ ) as white and black histograms for day and night activity; lower graphs give frequention rates (%) of each sole in the free (F), middle (M), shell (S), and oyster (O) zones.

Figure 5

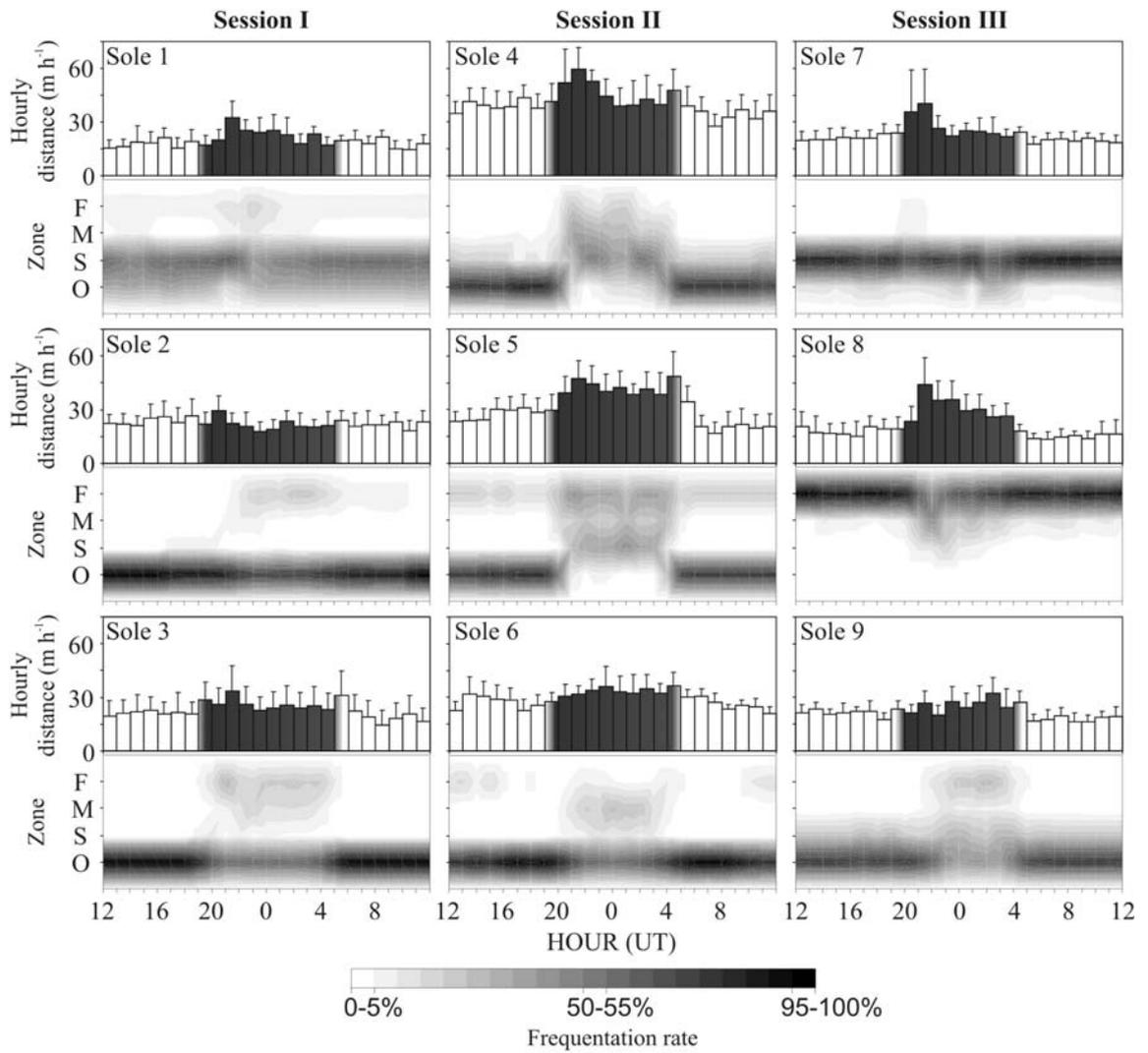
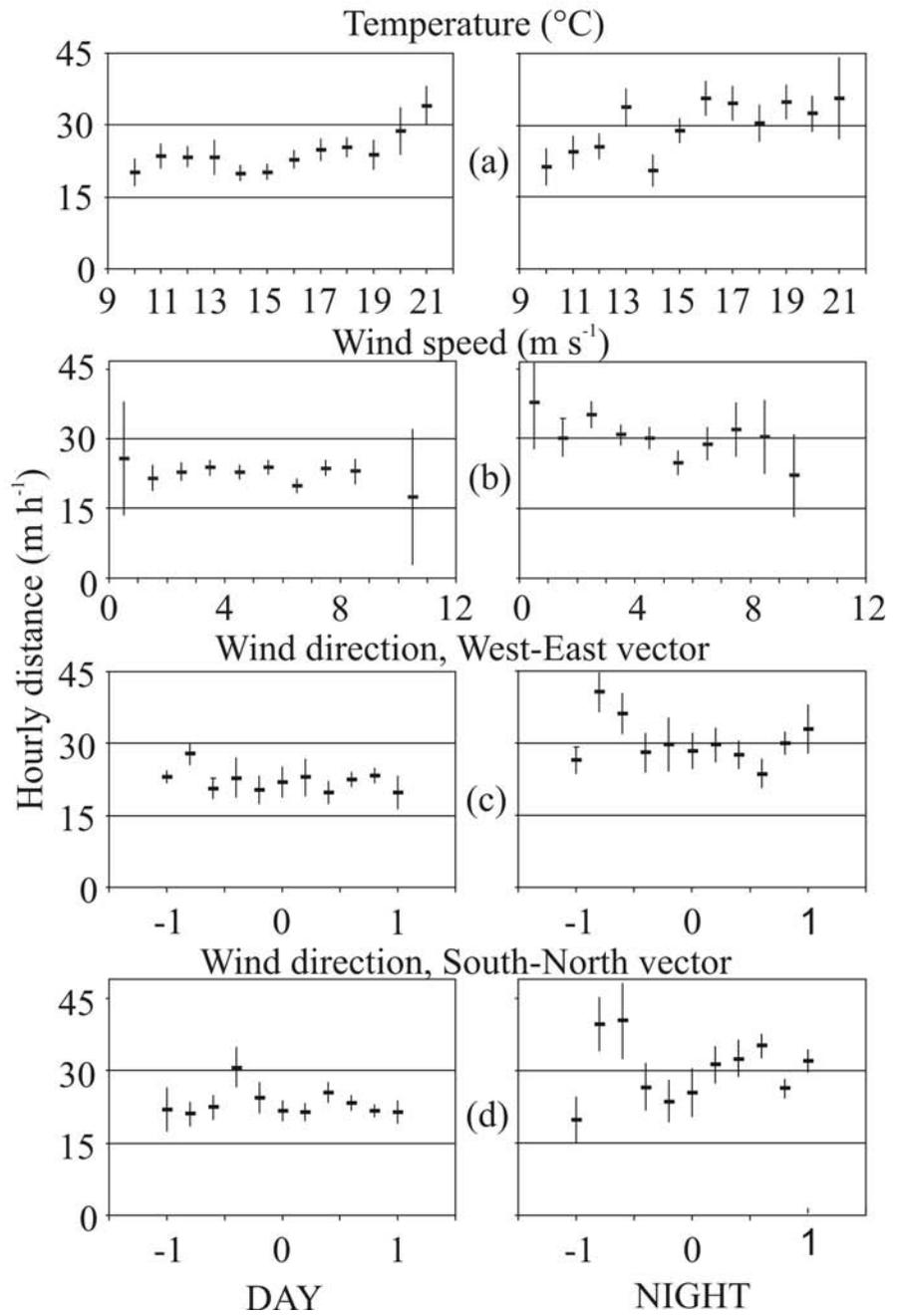




Figure 7. Changes in day-night swimming activities (mean hourly distances  $\pm$  s.d.), with (a) temperature, (b) wind speed, and (c and d) wind direction (west-east and south-north, respectively) as variable classes.

Figure 7



Running headings

*P. Laffargue et al.*

*Behaviour of sole in mesocosm with oyster-trestle cultures*

Table 1. The nine sole monitored.  $L_t$ , total body length,  $W_w$ , total body mass at initial / final states. M, Male, F, Female, and age (years).

Sole	$L_t$ (cm)	$W_w$ (g)	Sex / Age	Session
1	24.7 / 24.6	115.2 / 100.7	M / 2	I 3– 15 April
2	23.7 / 23.6	104.5 / 100.9	M / 2	
3	23.0 / –	91.9 / –	–	
4	23.2 / 23.3	89.4 / 85.9	F / 2	II 16–29 April
5	24.0 / 23.6	101.3 / 95.6	F / 2	
6	23.7 / 23.6	96.9 / 93.4	M / 2	
7	23.2 / 22.9	97.5 / 88.2	M / 2	III 29 April – 13 May
8	24.6 / 24.6	116.6 / 116.7	F / 2	
9	23.8 / 23.6	93.6 / 85.0	M / 2	

Table 2. Pearson correlation matrix (level of significance  $r = 0.07$  for  $p = 0.05$ ,  $n = 889$ ) calculated between hydrological variables (temperature, T°C; oxygen, O<sub>2</sub>; pH), meteorological data (wind speed; north-south wind direction, N-S wind, and east-west, E-W wind; atmospheric pressure; irradiance; rainfall), and water-column height in the pond (Water-col).

Parameter	O <sub>2</sub>	T°C	pH	Wind speed	N-S wind	E-W wind	Atmospheric pressure	Irradiance	Rainfall
O <sub>2</sub>	1								
T°C	0.24	1							
pH	0.35	0.44	1						
Wind speed	0.19	-0.28	-0.18	1					
N-S wind	-0.13	-0.22	0.30	-0.19	1				
E-W wind	-0.24	-0.34	0.17	-0.19	0.45	1			
Atmospheric pressure	0.13	0.56	0.30	-0.41	0.01	-0.14	1		
Irradiance	0.37	0.12	0.20	0.15	-0.19	-0.20	0.21	1	
Rainfall	-0.09	-0.09	-0.03	-0.03	-0.03	0.06	-0.15	-0.08	1
Water-column height	-0.01	0.05	-0.23	0.11	-0.13	-0.14	0.01	-0.04	0.043

Table 3. Nitrogen compound concentrations (mean  $\pm$  s.d. in  $\mu\text{g l}^{-1}$  and KW-ANOVA comparisons) in mesocosm water samples (nitrites  $\text{NO}_2$ , nitrites/nitrates complex  $\text{N}(\text{NO}_{2,3})$ , urea, ammoniac nitrogen  $\text{N}(\text{NH}_{3,4})$ ), and sedimentation rates (total dry sediment,  $\text{g m}^{-2} \text{d}^{-1}$ ), by (top) session, and (bottom) zone.

Parameter				KW-ANOVA
	Session I	Session II	Session III	( $n = 77$ or $78$ , d.f. = $2$ )
$\text{NO}_2$	$23.1 \pm 8.6$	$22.6 \pm 27.8$	$23.7 \pm 11.8$	$H = 6.9; p = 0.03$
$\text{N}(\text{NO}_{2,3})$	$1114 \pm 735.8$	$539.4 \pm 415.6$	$296.7 \pm 554.2$	$H = 24.9; p < 0.001$
Urea	$108.6 \pm 62.8$	$111.9 \pm 73.4$	$107.0 \pm 46.1$	$H = 0.7; p = 0.7$
$\text{N}(\text{NH}_{3,4})$	$37.0 \pm 17.1$	$27.1 \pm 14$	$37.1 \pm 21.1$	$H = 5.2; p = 0.08$
Sedimentation rate	$20.4 \pm 7.9$	$58.0 \pm 26.9$	$43.0 \pm 11.5$	$H = 16.6; p < 0.01$
				KW-ANOVA
	Oyster zone	Shell zone	Free zone	( $n = 77$ or $78$ , d.f. = $2$ )
$\text{NO}_2$	$32.6 \pm 24.7$	$16.5 \pm 9.7$	$20 \pm 8.7$	$H = 12.2; p = 0.002$
$\text{N}(\text{NO}_{2,3})$	$732.6 \pm 686.0$	$671.2 \pm 684.6$	$668.8 \pm 717.5$	$H = 0.8; p = 0.7$
Urea	$127.3 \pm 72.8$	$95.1 \pm 56$	$104.6 \pm 50.3$	$H = 6.9; p = 0.03$
$\text{N}(\text{NH}_{3,4})$	$45.8 \pm 21.9$	$28.5 \pm 11.3$	$27.3 \pm 12.5$	$H = 14.2; p = 0.001$
Sedimentation rate	$76.0 \pm 60.8$	$25.8 \pm 16.5$	$15.0 \pm 9.6$	$H = 37.1; p < 0.001$

Table 4. Comparisons between the three zones and the initial/final states of sediment quality (mean  $\pm$  s.d. of redox potential and sediment water content, SWC, g water / g sediment).

		Oyster zone	Shell zone	Free zone	KW-ANOVA
Redox potential (mv)	Initial state	22.8 $\pm$ 44.5	33.2 $\pm$ 43.2	29.1 $\pm$ 52.7	$H = 1.6$ ; $p = 0.45$ d.f. = 2; $n = 159$
	Final state	-17.4 $\pm$ 32.6	-5.6 $\pm$ 61.1	- 17.3 $\pm$ 24.5	$H = 0.5$ ; $p = 0.77$ d.f. = 2; $n = 162$
	U-test	$U = 2\ 088$ ; $p < 0.001$ d.f. = 1; $n = 106$	$U = 2\ 245$ ; $p < 0.001$ d.f. = 1; $n = 108$	$U = 2\ 229$ ; $p < 0.001$ d.f. = 1; $n = 107$	
SWC (%)	Initial state	67.1 $\pm$ 4.8	65.7 $\pm$ 4.1	66.3 $\pm$ 3.9	$H = 1.2$ ; $p = 0.56$ d.f. = 2; $n = 54$
	Final state	69.3 $\pm$ 3.7	65.6 $\pm$ 4.0	66.5 $\pm$ 3.5	$H = 8.5$ ; $p = 0.01$ d.f. = 2; $n = 54$
	U-test	$U = 86$ ; $p = 0.02$ d.f. = 1; $n = 36$	$U = 197$ ; $p = 0.27$ ; d.f. = 1; $n = 36$	$U = 156$ ; $p = 0.85$ ; d.f. = 1; $n = 36$	

Table 5. Between-sole comparisons of average activity by day and night. Within each group (I–V), sole activity was not significantly different.

Sole by day			Sole by night		
KW-ANOVA: $H = 238.7$ ; $p < 0.01$ $n = 1487$ ; d.f. = 8			KW-ANOVA: $H = 173.7$ ; $p < 0.01$ $n = 1068$ ; d.f. = 8		
Number	Mean rank	Group	Number	Mean rank	Group
8	516.2	I	2	388.8	I
1	586.3	I   II	1	412.2	I   II
3	638.5	I   II   III	9	453.1	I   II
9	661.1	I   II   III	7	454.5	I   II
7	682.9	II   III	3	461.3	I   II
2	746.9	III	8	527.7	II   III
5	781.7	III   IV	6	604.0	III   IV
6	925.3	IV	5	706.8	IV   V
4	1102.6	V	4	755.2	V