La mer 28: 248–254, 1990
Société franco-japonaise d'océanographie, Tokyo

Experimental study of swimming activity and orientation of sole (*Solea solea* L.) larvae and juveniles: influence of endogenous rhythm, light, gravity, temperature and feeding*

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Abstract: An experimental study of the swimming activity of sole larvae and juveniles was carried out to assess the migratory mechanisms of sole between the spawning areas, often located offshore, and the inshore and estuarine nurseries.

The experiments were carried out in different types of tanks fitted with special devices inducing sudden or progressive variations of external factors. Animal detection was achieved by photoelectric or optoelectronic barriers as well as by visual observations and an infrared converter.

The effects of endogenous rhythm, light, gravity, temperature and feeding are described. Experimental results are compared with *in situ* observations.

1. Introduction

In most areas of the French Atlantic coast and in particular in Biscay Bay, sole (*Solea solea*) spawning areas are located in the open sea but old larvae and early juvenile are mainly found in inshore areas and estuaries.

Hypotheses based on passive drift and spreading processes are inadequate to explain sole transport because of local currents parallel to the coast. Therefore, very likely active vertical and horizontal movements, are involved.

In the framework of the National Programme on the Recruitment Determinism (PNDR), a project focussed on the migratory mechanisms of sole larvae and juveniles was set up to try to predict the recruitment. The effects of internal factors (endogenous rhythm, age) and external factors (light, gravity, pressure, temperature, salinity, sediment, currents, feeding) on the swimming activity and the orientation of larvae and juvelile were experimentally studied. In this paper will be summarized the effects of endogenous rhythm, light and gravity, temperature and feeding.

2. Materials and methods

Most animals came from hatcheries, and a few were caught in the sea. Reared in the laboratory at constant temperature under natural illumination, sole were fed, according to their age on live *Artemia nauplii*, or dead *Artemia*, or pieces of chopped polychaetes. During the experiments, to maintain standard conditions and avoid changes in feeding behaviour, animals were not fed. Experiments concerning the effects of light, gravity and temperature were carried out on the different larval and juvenile stages from hatching to a size of about 40 mm. Experiments dealing with feeding behaviour were performed on metamorphosing larvae and metamorphosed soles.

In short duration experiments, visual observations were done, directly at strong light intensities or with an IR converter under very weak light intensities. In long duration experiments, automatic recordings using actographs and actotaxigraphs based on photoelectric cell detection (MACQUART MOULIN, 1979: CASTELBON, 1987) were made. The experiments involved different types of tanks-cylindrical, U-shape or parallelipipedic tanks-fitted with special devices to vary parameters (MACQUART-MOULIN *et al.*, 1976; MACQUART-MOULIN *et al.*, 1989; CASTELBON, 1987; CHAMPALBERT *et al.*, *in* press). Twotypes of light sources were used: a source giving a beam of parallel rays of white light and a fluorescent tube. Variations of the light intensity were achieved by means of neutral filters.

Data were treated and results expressed on different types of curves: actograms showing

*Received November 30, 1990

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hourly variations of activity; periodograms derived from WILLIAM and NAYLOR periodogram method (1972) or WHITTAKER and ROBINSON (1926) method; actotaxigrams describing spatio-temporal variations of soles as a function of time (CASTELBON, 1987).

3. Results

3. 1 Endogenous rhythm

In total darkness, stage I larvae did not exhibit a specific rhythmic swimming activity. Subsequently, a circadian rhythm appeared. Most often diurnal activity was high in stage 2 but early during ontogenesis (generally from stage 3), it became mainly nocturnal; from metamorphosis the main peak of swimming activity was consistently nocturnal (Fig. 1).

3. 2 Light and gravity

Due to the interrelated effects of light and gravity on the swimming activity and the migrations of marine animals, two types of experiments were carried out. Experiments in horizontal tanks were performed to study the effects of light on horizontal movements. Experiments in vertical tanks were done to study the effects of light, combined or not to those of gravity, on vertical movements.

Effects of light on horizontal movements; phototaxis in a horizontal plane

Orientation (phototaxis) and activity (photokinesis) were studied during short and long duration experiments on dark and light-adapted animals. This was done in horizontal tanks, light coming in laterally. A phototaxis index was calculated: 0 indicates the neutrality, values comprised between -1 and 0 a negative phototaxis and values between 0 and $+1$ a positive phototaxis. Photokinesis was expressed by an index corresponding to the percentage of soles reaching the wall closest to or farthest from the light source within 10 minutes.

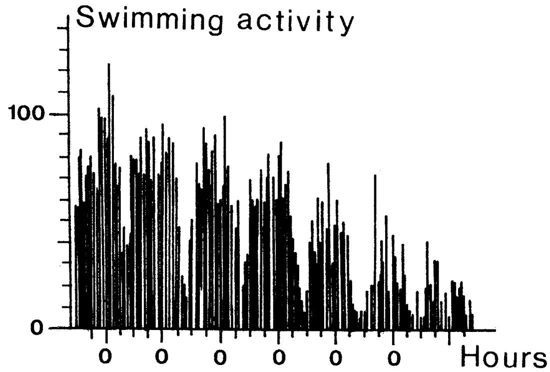

Fig. 1. Swimming activity of juvenile sole kept in total darkness

Moreover, to ascertain a possible role of a phototaxis rhythm, the experiments were carried out at different hours during the day and at night. No obvious rhythm of phototaxis appeared during the daily cycle in larvae. Nevertheless, from metamorphosis until a size of about 30 mm phototaxis index values were maximum for crepuscular or nocturnal illuminations. Figure 2 represents the phototaxis index variations during development. Up to an age of 2 or 3 days, larvae were neutral; then they remained photopositive at diurnal and nocturnal light intensities except for a few days before metamorphosis. During this short period of development, phototaxis decreased at diurnal illumination. From 35 mm, juveniles tended to be photonegative at diurnal illuminations.

The very low kinesis observed at diurnal intensities in larvae after hatching increased in the subsequent developmental stages. Just before metamorphosis there was a marked photoinhibition at diurnal intensities; from then, kinesis became maximum at crepuscular and nocturnal intensities (Fig. 2).

Effects of light and gravity on vertical movements

The effects of gravity and therefore geotactic responses were studied during short and long duration experiments in vertical tanks subjected to natural or artificial light variations. Swimming activity was low during the day. When light intensity was reduced artificially, whatever the time, the number of geonegative reactions increased; consequently an increased number of animals swam near the surface (Fig. 3). The reverse reaction was true: an artificial increase of light intensity reduced the geonegative reactions and increased the geopositive ones. The threshold of the geotaxis reversal varied with age. Close to

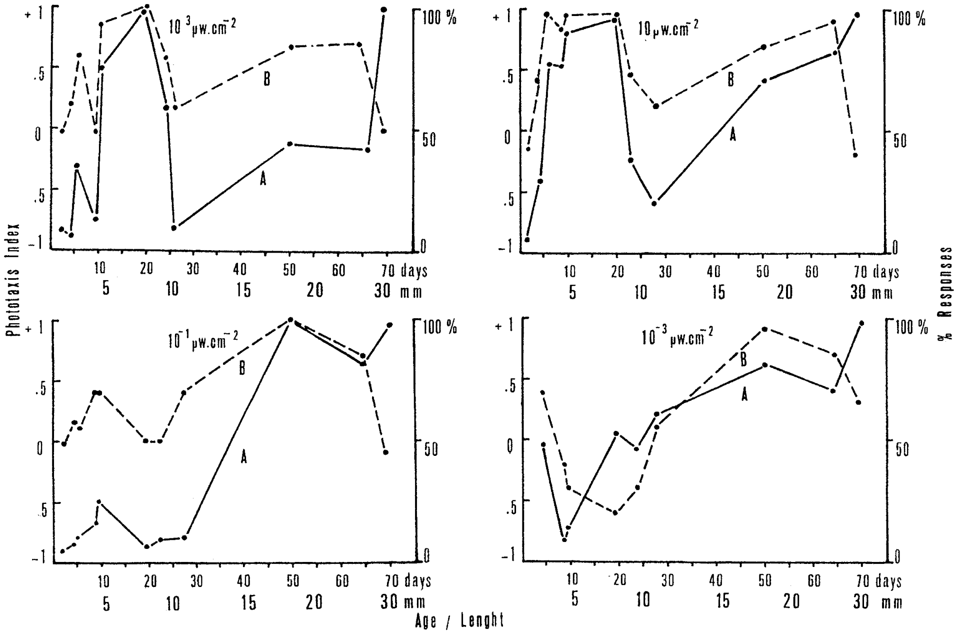

Fig. 2. Evolution of phototaxis and photokinesis of larvae and juvenile sole at diurnal (10^3 μW • cm^{-2}), crepuscular (10 and 10^{-1} μW • cm^{-2}) and nocturnal (10^{-3} μW • cm^{-2}) intensities (from CHAMPALBERT *et al.* in press)

10^{-1} μw•cm^{-2} one week after hatching, it was about 10^{-2} μw•cm^{-2} in 12-day-old larvae and reached 10^{-4} μw•cm^{-2} in young juveniles; in older soles (30 mm long) the threshold decreased to values as low as 10^{-6} to 10^{-7} μw•cm^{-2}.

The influence of kinetic variations induced by light and endogenous rhythm was demonstrated in juveniles subjected for several days to natural day-night variations or artificial

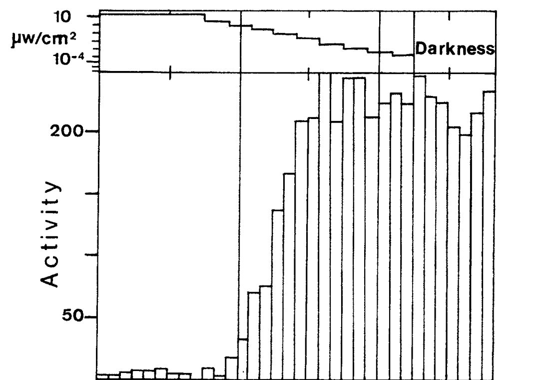

Fig. 3. Swimming activity variations induced by light intensity variations.

DL cycles of a 24 h periodicity, phased or inversely phased with the local natural cycle: in these conditions there was an obvious 24 h periodicity with a maximum activity during the dark phase. Figure 4 represents juvenile swimming activity as a function of time in an inverse DL cycle (f=12): There was little or no activity during the light phase (from 6 p.m. to 6 a.m.) and high activity with a main accumulation of juvenile near the surface during the dark phase (from 6 a.m. to 6 p.m.).

The respective effects of geotaxis and phototaxis on orientation reactions were determined in experiments carried out either in U-shape tanks illuminated from one of their surfaces or in cylindrical vertical tanks illuminated laterally or from below.

Figure 5 (top) shows the actogramme of juvenile sole activity respectively near the surface and near the bottom when animals were kept in a cylindrical tank illuminated from below with natural light. During the night swimming activity was low near the bottom and high near the surface. At nocturanal intensities, positive phototaxis should

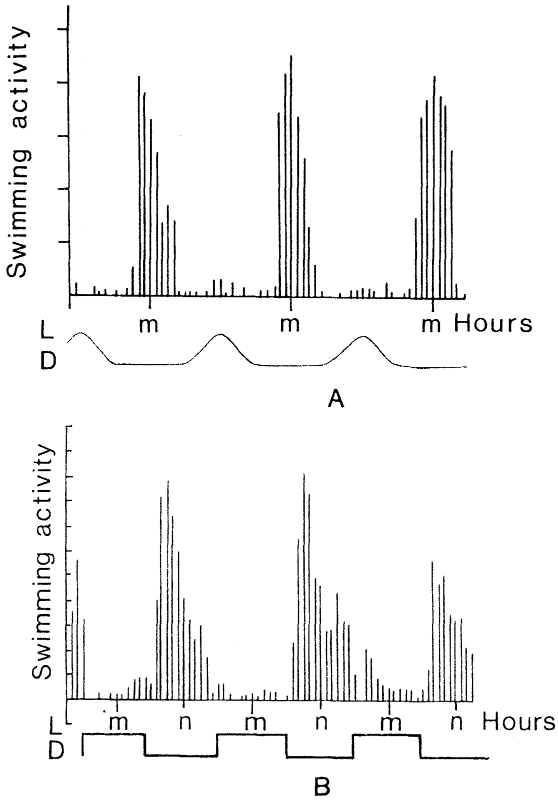

Fig. 4. a) Actogram of juvenile sole in artificial light phased with the local natural cycle; experiment with progressive changes in light intensity.
b) Actogram of sole juvenile in artificial light inversely phased with the local natural cycle; experiment with sharp changes in light intensity.

keep animals in the lower part of the tank. Therefore, the accumulation near the surface was induced by geonegative reactions only; phototaxis and geotaxis were thus antagonistic.

Figure 5 (bottom) shows the activity of sole swimming near the surface in a U-shape tank illuminated from one of the two surfaces. At night, the activity near the surface was higher in the illuminated part of the tank than is the dark one, thus demonstrating the combined effects of geotaxis and phototaxis.

3. 3 Temperature

Three types of experiments were carried out on juveniles: experiments at constant temperatures, experiments with temperature gradients; experiments with cyclic variations of temperature. The effects of temperature on swimming activity, endogenous rhythm and distribution were considered and the combined effects of light and temperature subsequently studied. Activity was increased at high temperatures within the tolerated range. In total darkness, juveniles subjected to 12 h temperature cycles exhibited a rhythmic activity of the same period. Activity increased during the heating phase, and decreased during the cooling phase, whatever the time of day (Fig. 6).

Juveniles subjected to both temperature variations and diurnal light variations tended to exhibit a 24 h rhythmic activity thus demonstrating the major effect of light compared to temperature.

In experiments with temperature gradients, juveniles tended to avoid the warmest surface layer in total darkness as well as in DL conditions. This was particularly obvious when the range of variations was wide.

3. 4 Feeding

The effects of food types and concentrations on swimming activity and fixation of juvenile soles were studied in total darkness and under natural DL conditions. Animals were given different types of food: live pelagic (*Artemia naupii*) or benthic (polychaetes) food, or frozen food (nauplii or adults of *Artemia*, or polychaetes).

About one hour after feeding swimming activity of juvenile sole decreased markedly, but more or less noticeably according the food concentrations they were given. This occurred in DL conditions, as well as in total darkness. Swimming stopped after every feeding and resumed from 1 to 4 days later. Similar results were obtained with live *Artemia nauplii* or polychaetes. No decrease in activity was observed with dead food in animas used to feeding on live food.

4. Discussion and conclusions

Our experiments clearly demonstrate the existence of endogenous rhythm in old larvae and juveniles soles and confirm previous results concerning old juveniles (Champalbert and Castelbon, 1989). This agrees with

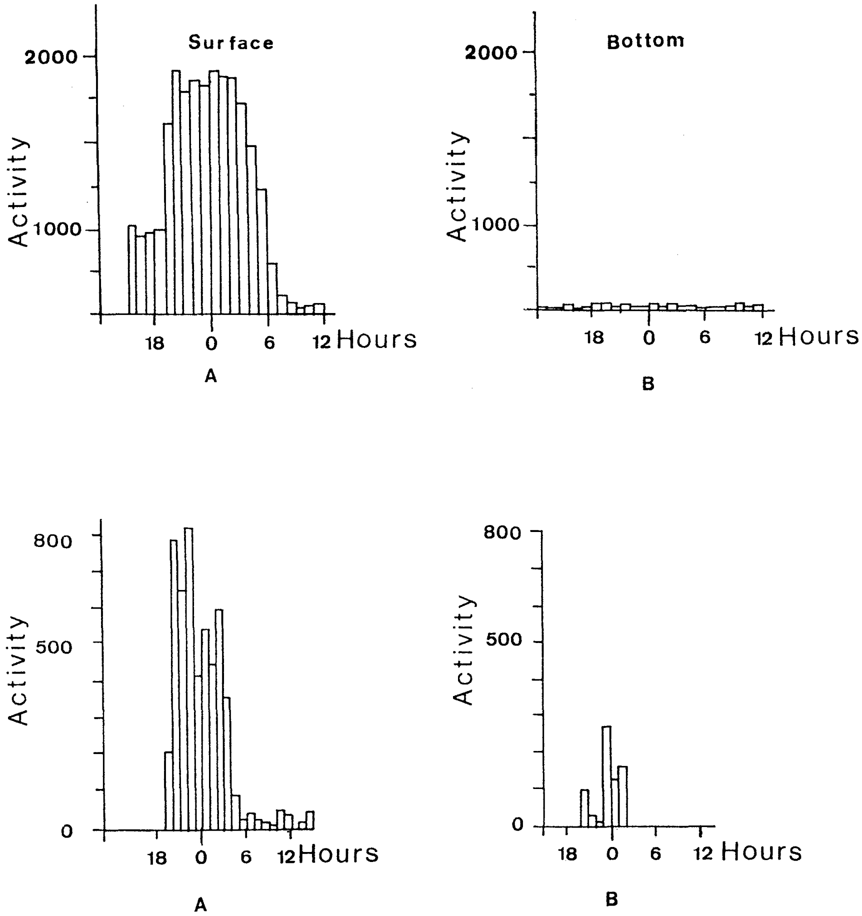

Fig 5. top: Actogram of juvenile sole activity near the surface (A) and the bottom (B) of a cylindrical tank illuminated from below.
bottom: Actogram of juvenile sole acfivity near the surface of a U-shape tank.
A: activity near the dark surface; B: activity near the illuminated surface.

results on flatfish (O' Connor, 1972; GIBSON, 1973, 1976).

In old larvae as well as in juveniles the external factors can modify the endogenous circadian rhythm of swimming activity. Some of these factors such as temperature, induce behaviour changes but cannot synchronize a rhythm. Our experiments showed that sole migrations are not prevented by temperature gradients but animals tend to avoid high temperatures. In the same way, CHAMPALBERT *et al.* (in prep.) observed that salinity gradients do not constitute a real barrier to vertical movements. These results agree with the penetration of animals into estuarine nurseries and coastal waters where temperature and salinity vary widely (MARCHAND *et al.*, 1989).

Light and gravity play a greater role than temperature and salinity, in the control of migratory processes of larvae and juvenile soles. The importance of phototaxis reactions on herring and flatfish larvae, noted by BLAXTER (1969, 1972) was confirmed by our experiments on sole (CHAMBALBERT *et al.*, in prep.). We also observed that geotaxis, phototaxis and photokinesis have combined or antagonistic actions which vary during development. Important behaviour changes occur a few days after hatching, then during metamorphosis and lastly above a size of 35 mm.

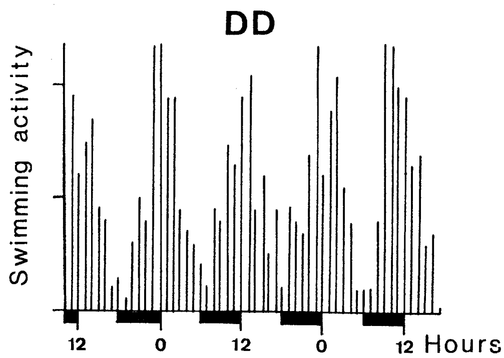

Fig. 6. Swimming activity of juvenile soles subjected in total darkness to 12 htemperature cycles. Dark stripes indicate the heating phases.

From this size on, juvenile behaviour did not seem to differ from the adult one described by KRUUCK (1963).

Our experimental sutdies, in agreement with field observations, can explain some migratory processes and characteristic changes of distribution during the ontogenesis. For a few days after hatching (3–4 d) whatever the intensity, geotaxis is positive, phototaxis neutral and photokinesis low. This may explain why early larvae are found in deep waters. In larvae from 7 days to before metamorphosis the number of geonegative increases at diurnal intensities. At this stage, photokinesis is maximum and phototaxis positive. Such behaviour may be responsible for the progressive upward migrations observed in old larvae in the sea. From early metamorphosed to larger juveniles (30–35 mm), the number of geonegative reactions and photokinesis decrease at diurnal intensities; they are maximum at crepuscular or nocturnal intensities. Except in the first stage and metamorphosing larvae, phototaxis remains positive and one can assume that it acts in the horizontal plane, inducing movements towards illuminated shallow areas; its action in the vertical plane is possible only for illuminations lower than the geotaxis inversion threshold. In the sea this juvenile period corresponds to a migration towards the bottom. Finally, positive geotaxis, negative phototaxis and low kinesis characteristic of old juveniles behaviour in the laboratory, agree with the decrease of migration amplitude observed in the sea.

In addition to hypotheses based on physical mechanisms and spreading by diffusion (KOUTSIKOPOULOS *et al.* in press) our experiments give new evidence to explain the transport of sole larvae from offshore to inshore areas. Oriented movements and increased activity of juveniles that metamorphose in offshore areas, could enhance the spreading as a result of decreased activity in coastal areas along with a decreased kinesis juveniles may remain temporarily in estuaries.

REFERENCES

BLAXTER, J. H. (1969): Visual thresholds and spectral sensitivity of flatfish larvae. J. exp. Biol., **51**: 221–230.

BLAXTER, J. H. (1972): Brightness discrimination in larvae of plaice and sole. J. exp. Biol., **57**: 693–700.

CASTELBON, C. (1987): Les migrations nycthémé rales du zooplancton. Déterminisme expérimental des réactions locomotrices. Thése d'Etat, Univ. Aix-Marseille 2, 258 p.

CHAMPALBERT, G. and C. CASTELBON (1989): Swimming activity rhythms in *Solea vulgaris* (Q.) juveniles. Mar. Behav. Physiol., **14**: 201–209.

CHAMPALBERT, G., A. BOURDILLON, C. CASTELBON, D. CHIKHI, L. LE DIREACH-BOURSIER, C. MACQUART-MOULIN and G. PATRITT (1989a): Déterminisme des migrations des larves et juvéniles de soles. Océanis, **15**(2): 171–180.

CHAMPALBERT, G., C. CASTELBON, L. LE DIREACH-BOURIER and A. BOURDILLON (1989b): Locomotory activity rhythms in juvenile *Solea vulgaris* Q. and *Scophtalmus maximus* L. (Abstract). Rapp. P. V. Reun. Cons. int. Explor. Mer, **191**: 400–408.

CHAMPALBERT, G., C. MACQUART-MOULIN, G. PATRITI and D. CHIKHI (in press): Ontogenic fluctuations of the phototaxis in larvae and juvenile sole *Solea solea* L. J. exp. mar. Biol. Ecol.

GIBSON, R.N. (1973): Tidal and circadian activity rhythms in juvenile Plaice, *Pleuronectes platessa*. Mar. Biol., 22: 379–386.

GIBSON, R.N. (1976): Comparative studies on the rhythms of juvenile flatfish. *In*: Biological rhythms in the marine environment (P. J. DECOURUS, ed.) Univ, South Carolina Press, Columbia, South Carolina, USA. pp. 199–213.

KOUTSIKOPOULOS, C., M. KARAKIRI, Y. DESAUNAY and D. DOREL (1989): Response of sole juveniles to abrupt environmental changes in the bay of Vilaine (Bay of Biscay). Early life history of fish symposium, Bergen, Oct. 1988. Rapp. P. V. Reun. Cons. int. Explor. Mer. 91.

Koutsikopoulos, C., L. Fortier and J. Gagne (in press) : Cross-shelf dispersion of Dover Sole eggs and larvae (*Slea solea*) in Biscay Bay and recruitment to inshore nurseries. J. Plankton Res.

Kruuck, H. (1963): Diurnal periodicity in the activity of the common Sole, *Solea vulgaris* Quensel. Neth. J. Sea. Res., **2**, 1–28.

Macquart-Moulin, C. (1976): Rythmes d' activité persistants chez les Peracarides du plancton nocturne en Méditerranée (Amphipodes, Isopodes). Mar. Behav. Physiol., **4**: 1–15.

Macquart-Moulin, C. (1979): Ecophysiologie comparée des Mysidacées *Hemimysis speluncola* Ledoyer (cavernicole) et *Leptomysis lingvura* G.O. Sars (non cavenicole). L' orientation a la lumiére: tests ponctuels. J. exp. mar. Biol. Ecol., **38**: 287–299.

Macquart-Moulin, C., C. Castelbon, G. Champalbert, D. Chikhi, L. Le Direach-Boursier and G. Patrti (1989): The role of barosensitivity in the control of migrations of larvae and juvenile Sole (*Solea vulgaris*): influence of pressure variations on swimming intensity and orientation. Early life history of fish symposium, Bergen, Oct. 1988. Rapp. P. V. Reun. Cons. int. Explor. Mer., **191**: 400–408.

Marchand, J., G. Marchand and G. Masson (1989): Process of estuarine colonization by Sole (*Solea solea*): hydrological conditions, behaviour and feeding activity in the Vilaine estuary (France). Early life history of fish symposium, Bergen, Oct. 1988. Rapp. P. V. Reun. Cons. int. Explor. Mer., **191**: 287–295.

O'connor, J.M. (1972): Tidal activity rhythm in the Hogchoker *Trinectes maculatus*. J. exp. mar. Biol. Ecol., **9**: 173–177.

Whittacker, E. and G. Robinson (1926): *In*: The calculus of observations. A treatise on numerical mathematics (Blackie & Son ltd., 4ème ed., 1965), London, Glasgow, 397 p.

Williams, J. A. and E. Naylor (1978): A procedure for the assessment of significance of rhythmicity in time-series data. Int. J. Chronobiol., **5**: 435–444.

Résumé: Une étude expérimentale de l'activité locomotorice des larves et juvéniles de sole a été entreprise pour comprendre les mécanismes migratoires des soles entre les aires de ponte, souvent situées au large, et les nurseries cotières ou estuariennes.

Les expériences ont été effectuees dans différents types de cuves munies de dispositifs permettant de réaliser des variations soudaines ou progressive des facteurs extenrnes tels lumière, température, salinité, pression. La détection des animaux est faite à l'aide de barrages phototélectriques ou optoélectroniques ainsi que par observation visuelle directe ou avec un convertisseur infrarouge.

Les effets du rythme endogène, ceux de la lumière, gravité, température et alimentation sont résumés. Les résultats expérimentaux sont compares avec les observations obtenues dans le milieu naturel.