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Activity Rhythms of Barnacles as a Behavioural Bioindicator of Copper Pollution in Lake Timsah, Suez Canal, Egypt

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ABSTRACT

The present study aimed to introduce barnacles as a behavioural bioindicator in Lake Timsah, Ismailia, Egypt. A special design is successfully applied for automatical monitoring of the barnacle cirral activities. Two barnacle species, *Balanus eburneus* and *B. amphitrite*, were collected for the present experiments. The observed typical cirral activities served mainly for feeding. Fast beats reached up to 100 beats per minute whereas normal beats were up to 50 beats per minute. *B. eburneus* exhibited circatidal rhythmicity under free-running conditions (continuous darkness and constant temperature). As an indicator of the pollution level, the cirral activity patterns were observed under different copper concentrations. Exposure to Cu^{2+} concentrations of 3.5, 3, 2.5, 2, 1.5, 1, 0.8, 0.6, 0.4, and 0.2 mg/L was achieved to determine 24, 48, 72, and 96 hr LC_{50} values. The LC_{50} values were 1.33, 0.635, 0.40 and 0.291 mg/L in *B. eburneus* whereas they reached 1.184, 0.493, 0.272 and 0.139 mg/L in *B. amphitrite*, respectively. The *B. eburneus* cirral activity pattern was directly and proportionally influenced by water copper concentrations at 24 and 96 hr LC_{50} conditions. It could be concluded that the cirral activity rhythmicity in barnacles, as an essential behavioural pattern, may be considered as a good tool for monitoring the pollution in Lake Timsah. The developed design may also be a useful tool for studying several environmental interactions of the barnacles.
Key words: *Balanus eburneus,* cirral activity, marine environment, rhythmicity monitoring, toxicity.

INTRODUCTION

Barnacles are a very successful group of crustaceans that dominate particular zones of the rocky littoral areas in most parts of the world. They are amongst the most important fouling organisms in Lake Timsah (Ghobashy *et al.*, 2005). The barnacle is a filter feeder, taking food through the cirri into its mouth. A barnacle may use any of the three patterns of cirral activities: normal beat, fast beat, and extension (Pullen and LaBarbera, 1991). Normal beat generates a respiratory current and also results in food capture. In fast beat, cirri do not swing withdrawals into the mantle cavity as in normal beat. There is no water flow to the mantle, so no proper respiration, only food capture. In the prolonged cirral extension, cirri extend in a moderate water flow facing the current for food capture (Anderson, 1994). These endogenous rhythms of the cirri have been observed (Anderson and Southward, 1987; Trager *et al.,* 1992).

Biomonitoring of heavy metals is an important tool for assessing the degree of pollution in water. Various organisms are potentially useful as biomonitors, e.g. polychaetes, oysters, mussels, clams, and barnacles. Accumulating metals taken up from water, sediments, and food, biomonitors offer integrated measures of the supply of trace metals available in an environment (Rainbow, 2002). Recently, a great deal of attention was applied to barnacles as focal species for valuable trace metal monitoring (Zacharias and Roff, 2001; Rainbow *et al.,* 2002).

Copper is an essential trace metal for animals, but it is also among the most toxic metals to aquatic invertebrates. Antifouling ships' coatings containing copper are a major source for introducing copper into the marine and estuarine environments (Foerster *et al.,* 1999; Thomas *et al.*, 1999). In addition, a considerable amount of copper is contributed from organic wastes of industrial and agricultural origin (Naqvi *et al*., 1998). Lake Timsah receives pollutants mainly through the transport of ships via the Suez Canal. Additionally, concentrations of copper in barnacles from contaminated sites are among the highest of any marine organisms (Linthurst *et al.*, 1995; Rainbow, 1995 & 1997; Ruelas-lnzunza and Páez-Osuna, 1998; Rainbow *et al.*, 2000; Rinderhagen *et al.*, 2000; Rainbow and Blackmore, 2001; Rainbow and Wang, 2005).

The objectives of the present investigation are:
(1) monitoring of the barnacle's cirral activity by applying a special design with a computer-aided system (2) determination of the acute toxicity of copper on the tolerance of *Balanus eburneus*, and *B. amphitrite,* and (3) evaluating the cirral activity of *B. eburneus* as a behavioural bioindicator of pollution.

MATERIALS AND METHODS

Experimental animals and maintenance

Adult *Balanus eburneus* and *B. amphitrite* were collected from Lake Timsah. Active adults of similar ages (13±1 and 15±2 weeks) and uniform-sizes of rostro-carinal length (14±2 mm and 13±2 mm) for *B. eburneus* and *B. amphitrite*, respectively, were carefully placed in continuous and slightly aerated containers. Barnacles were acclimatized for two hours to laboratory conditions. They were fed on *Artemia* nauplii

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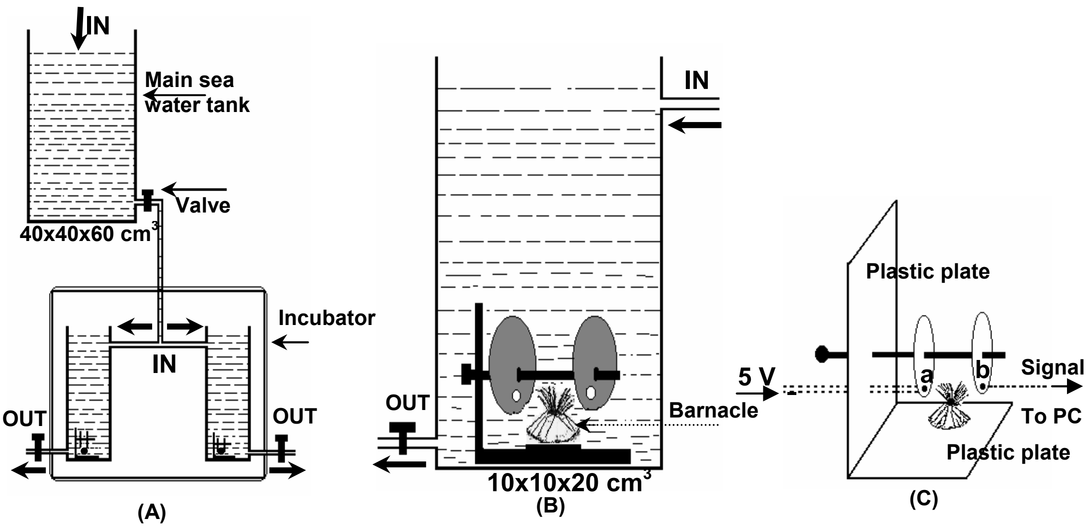

Figure (1): Schematic drawing illustrates (A) The renewal static flow system used during the experiments, (B) The design applied for recording the cirral activity patterns, and (C) Animal holder components: a) IR-LED, b) phototransistor, and PC) personal computer.

once daily. Using a static renewal system, water was changed every two days for toxicological, and every day for behavioural experiments (Fig. 1A&B). During the experiment, water salinity (35±1 ‰), temperature (20±2°C), and pH (7.9) were monitored in control and experimental containers.

Toxicity determination of copper

Static renewal standard bioassays were employed (FAO, 1977). Proper concentrations of copper sulphate [Cu (SO_4)] in distilled water were prepared and acidified with one ml acetic acid to prevent any precipitation in seawater. Groups of 12 specimens were exposed to Cu^{2+} concentrations for up to 96 hours. *B. eburneus* specimens were exposed to 3.5, 3, 2.5, 2, 1.5, 1, 0.8, 0.6, 0.4, and 0.2 mg/L. Those of *B. amphitrite* were exposed to 3.5, 2.5, 2, 1.5, 1, 0.4, and 0.2 mg/L. The mortality and cirral activity were observed for 24 hours. The LC_{50} was estimated by Abbott's formula and graphical analysis (Finney, 1971).

Monitoring of cirral activity patterns

(a) Mounting Design

The animal holder was composed of base and vertical plastic plates having a rectangular shape. An adjustable activity sensor was supplied. A photocell circuit of an IR-LED and a phototransistor were installed. All connectors were isolated by wax (Fig. 1C).

(b) Hardware setup

Experiments were run under the control of a DOS-1601 interface (Keithley Instrument, Inc.).
An integrated data acquisition and analysis package (Test Point ver. 1994, Capital Equipment Corporation) was used.

(c) Recording the activity

B. eburneus was very tolerant to copper. Therefore, it was selected for monitoring the cirral rhythmic activity. The experiment was conducted in an incubator under continuous darkness and ambient temperature of 28±1ºC. Each animal was separately mounted on the base plate so that its cirral movement could be detected upon interruption of the induced light beam. Sampling rate was every 5 minutes to check the cirri position. Animal watching continued for 24 hours.

RESULTS

The current observations revealed that adult barnacles' behaviour was limited in range. Under laboratory conditions, adult barnacles expressed cirral activities for feeding and respiration, metabolic activity indicated by the release of faecal pellets, protective withdrawal and closure, and partial extension and withdrawal of the furled cirri (pumping).

Tolerance and LC_{50} determination

The results obtained from mortality tests of copper are presented in figures (2) and (3). All control *B. eburneus* animals survived, whereas two *B. amphitrite* individuals died after 48 and 72 hours. The concentrations 0.2, 0.4 and 0.6 mg/L caused no mortality on the first day for *B. eburneus*. Mortality increased with increasing of both concentration and time of exposure. It reached 100% for 3.5 mg/L after 24 hours of exposure in both species. After 8 hours of exposure to the concentration 3.5 mg/L, eight animals died (66.6%). Whereas after 48 hours 100% mortality was reached by the concentrations of 1.5 mg/L and 2 mg/L for *B. eburneus* and *B. amphitrite*, respectively.

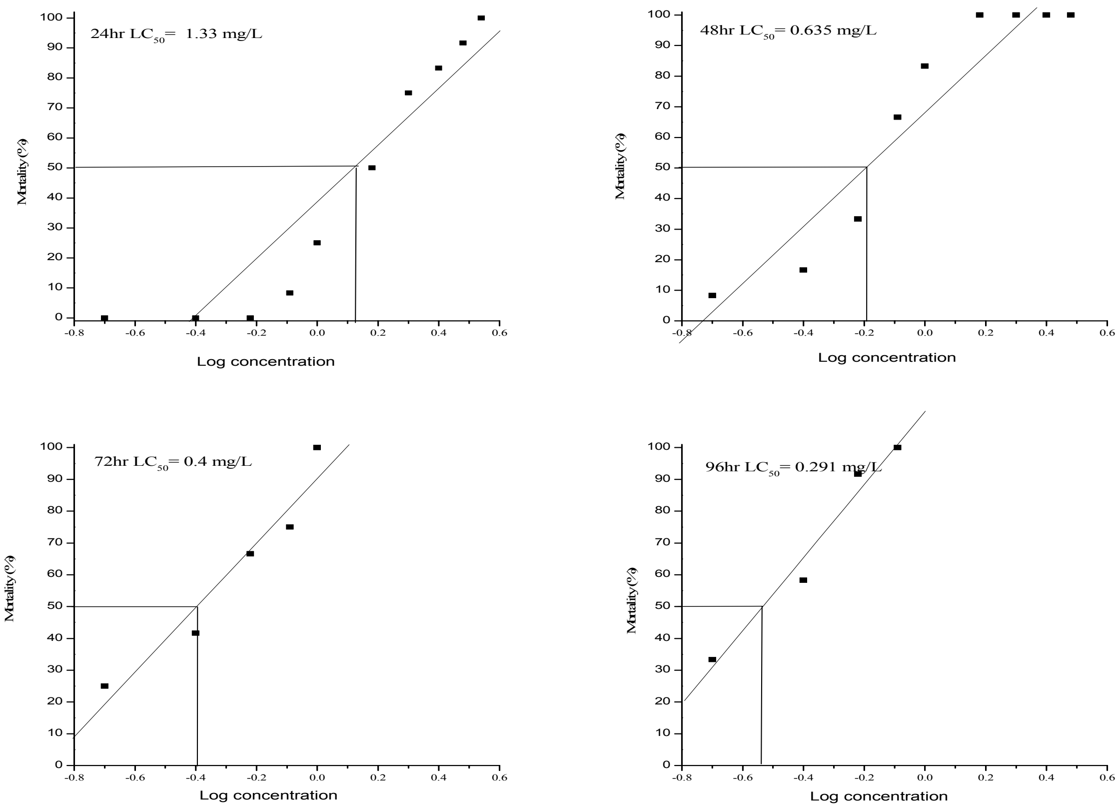

Figure (2): Levels of LC_{50} copper toxicity to *Balanus eburneus* adults after various copper exposure periods.

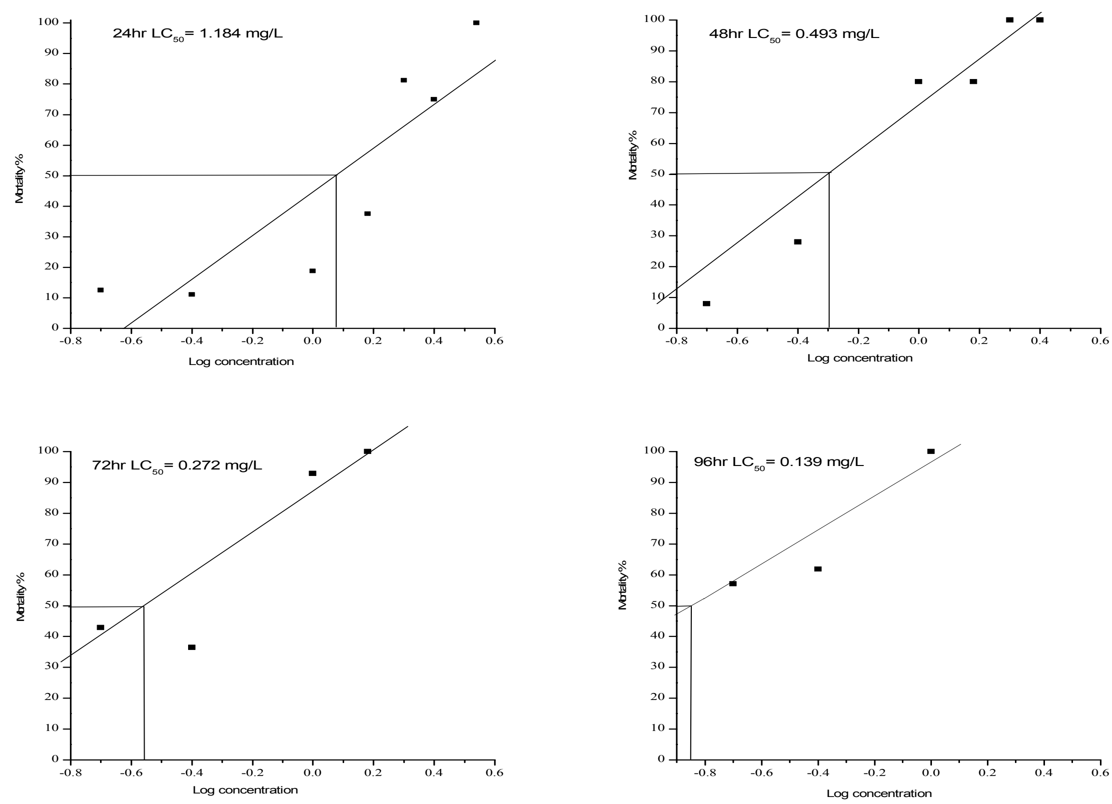

Figure (3): Levels of LC_{50} copper toxicity to *Balanus amphitrite* adults after various copper exposure periods.

After 72 hours, 100% mortality was reached by the concentrations of 1 mg/L and 1.5 mg/L for *B. eburneus* and *B. amphitrite*, respectively.

Control barnacles expelled two faecal pellets about once every 4 hours. Animals exposed to copper concentration of 0.1 mg/L showed more metabolic activity than animals in 0.2 mg/L and 0.4 mg/L tanks. Whereas more faecal pellets were released by the former, animals at 0.6 mg/L tank and higher concentrations ceased faecal pellet release during the experiment. The 24 hr LC_{50} treated animals expelled no faecal pellets whereas the 96 hr LC_{50} treated animals expelled only one faecal pellet per day. Animals before dying showed a low rate of cirral activity (weak normal beat or weak pumping beat) until cirri were completely inhibited in an outward position at the tip of the shell aperture, which was slightly opened. The animals' response before dying was very weak and their closure defence reaction was almost absent.

The estimated LC_{50} values after exposure to copper concentrations for 24, 48, 72 and 96 hours reached 1.33, 0.635, 0.40 and 0.291 mg/L for *B. eburneus,* whereas it reached 1.184, 0.493, 0.272 and 0.139 mg/L for *B. amphitrite*, respectively.

Cirral activity rhythms

Naturally, barnacles showed cirral activity; the cirri extend and then withdraw either into the mantle cavity (normal beat) or incompletely into the mantle cavity (fast beat). Adult *B. eburneus* exhibited a probably endogenous circatidal cirral rhythm, related to the tide cycles, under free-running conditions of constant temperature and continuous darkness (Fig. 4A). A circatidal period of about 24.7 hours was observed in the activity of *B. eburneus*. No superimposed circadian rhythm was confirmed in the individuals under investigation.

In general, two modes of cirral activity were recognized, namely active and resting modes. The active phase was represented by both normal and fast beating frequencies. Figure (4A) shows that fast beat was observed during the second half of the subjective day whereas normal beat occurred during the first half of the subjective day. The fast beat activity lasted longer than the normal beat. For control animals, maximum observed fast beat per minute was 120 beats and the lowest normal beat was 32 beats. As illustrated in figure (4 B&C), the fast beat frequency range was 80-100, whereas the normal beat was 30-50 beats/minute. Fluctuations between the observed high and low limits of fast and normal beats lasted between 75-90 minutes and 40-60 minutes, respectively. The extension mode of cirral activity, where barnacles simply hold the cirral basket against the water flow, could not be distinguished during this automated recording but was occasionally noticed through personal animal watching.

Cirral activity patterns under copper exposure

As illustrated in figures (5) and (6), exposure to copper seriously influenced cirral activity. Upon exposure to copper, barnacles exhibited no clear patterns. Additionally, fast beat was not recorded in the treated animals. Exposure to 24 hr LC_{50} yielded a descending normal beat pattern (Fig. 5). Before dying, animals showed few slow beats during the last 6-9 hours. Under 96 hr LC_{50} exposure, however, individuals exhibited scattered normal beat till the second day (Fig. 6). Lower beat rates followed by longer resting times were frequently observed. No normal beat was recorded during the 4^{th} day.

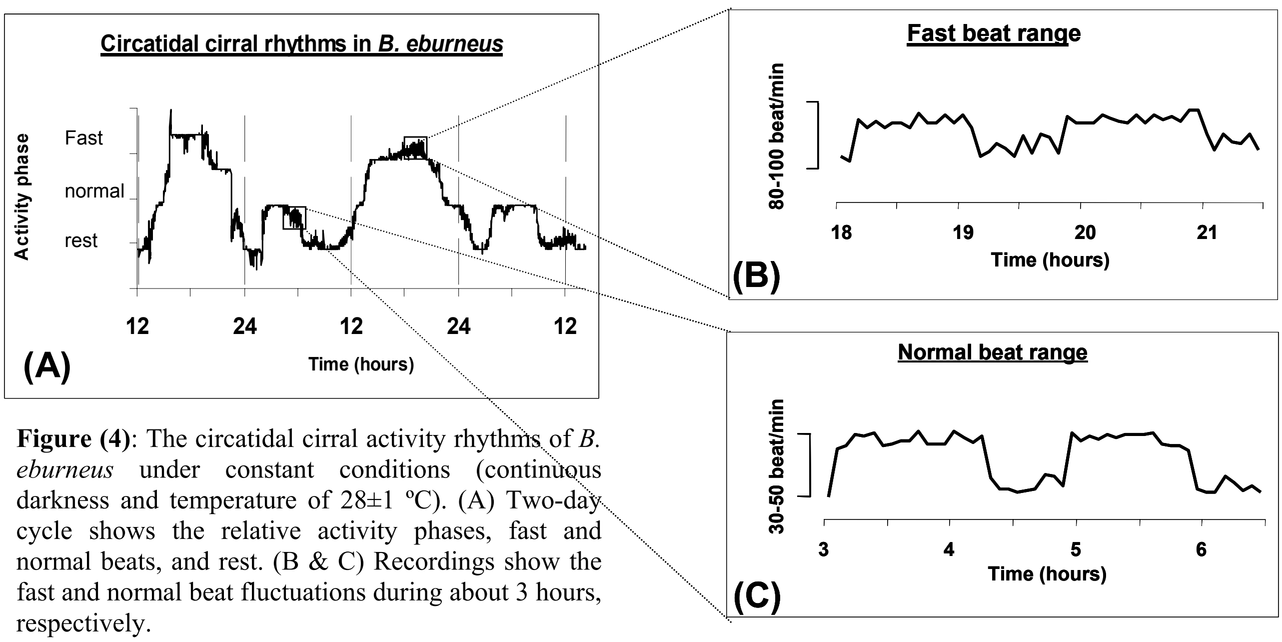

Figure (4): The circatidal cirral activity rhythms of *B. eburneus* under constant conditions (continuous darkness and temperature of 28±1 ºC). (A) Two-day cycle shows the relative activity phases, fast and normal beats, and rest. (B & C) Recordings show the fast and normal beat fluctuations during about 3 hours, respectively.

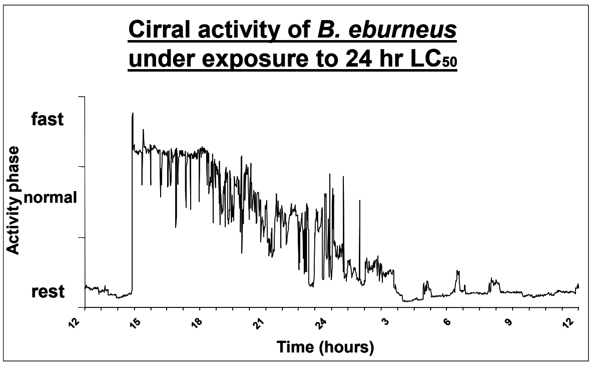

Figure (5): The effect of exposure to 24 hr LC_{50} of copper on the cirral activity of *B. eburneus* under constant DD and temperature of 28±1 ºC.

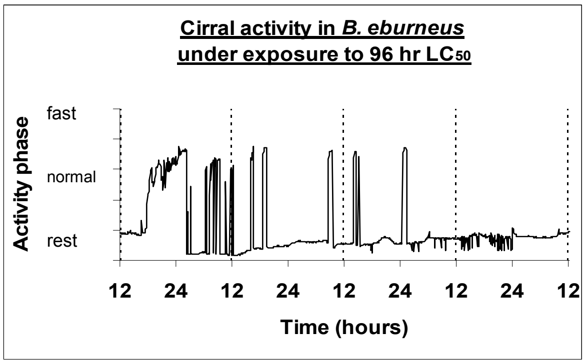

Figure (6): The effect of exposure to 96 hr LC_{50} of copper on the cirral activity of *B. eburneus* under constant DD and temperature of 28±1 ºC.

DISCUSSION

Different impacts of copper as a pollutant on biological activities of aquatic organisms may be observed, e.g. feeding behaviour, physiological and morphological changes, and mortality. The current study focused on cirral activity patterns during copper exposure.

Copper clearly exhibits a lethal effect on barnacles and may be considered highly toxic. This study reveals that 48 hr LC_{50} values reached 635 and 493 µg/L for *B. eburneus* and *B. amphitrite,* respectively. Kamrin (1997) reported that metals with LC_{50} lower than 100 µg/L are considered very highly toxic, 100-1000 µg/L are considered highly toxic, and 1000-10000 µg/L are considered moderately toxic. Toxicity is related to a threshold concentration of metabolically available metal and not to total accumulated metal concentration (Rainbow, 2002). Toxicity experiments on adult *B. eburneus* and *B. amphitrite* indicated that increasing copper concentration and exposure time would increase mortality rate. Similar results were obtained with barnacles (Powell and White, 1990; Rainbow *et al.,* 1993). In the present investigation, the 96 hr LC_{50} values reached 291 and 139 µg/L for *B. eburneus* and *B. amphitrite* adults, respectively. On the contrary, lower LC_{50} for *B. improvisus* 140, 110 and 100 µg/L were recorded for 12, 24, and 48 hr exposures, respectively (Irwin *et al.,* 1997). Also barnacle *B. amphitrite* larvae showed LC_{50} values ranging between 145-213 µg/L (Qiu *et al.,* 2005). However, individual response of the adults to toxicants may be species-specific.

About 51 toxicity studies were conducted on *B. amphitrite*, while only 21 on *B. eburneus* (Orme and Kegley, 2002). Therefore, in addition to its tolerance to copper pollution, cirral activity monitoring was focused on *B. eburneus*. Laboratory observations confirmed the importance of the cirral appendages as a function for barnacle behavioural activities. Barnacles feed by extending the cirri into the water flow. The cirri length is plastic; the higher the water velocity, the shorter the cirrus (Arsenault *et al.,* 2001; Natasha and Denny, 2004). The cirri may also regenerate (Harvey *et al.,* 2003). Individuals under investigation had similar ages (13±1 weeks) and were exposed to constant temperature and the same posture on the mounting plate in a static flow system. Different parameters could affect cirral activity behaviour patterns, e.g. temperature (Thiyagarajan *et al.,* 2003), age being faster in young than older animals (Anderson, 1994), tidal level and water flow (Trager *et al.,* 1990; Marchinko and Palmer, 2003; Benny *et al.,* 2005), and orientation of the barnacle itself (Pasternak *et al.,* 2000).

The present approach is the first attempt for a detailed analysis of the cirral activity patterns in barnacles using an automated computer system. The applied design has successfully proven the presence of different cirral activity patterns, fast beat and normal (slow) beat. These types were also mentioned by Pullen and LaBarbera (1991), and Achituv and Yamaguchi (1997). Fast beat is considered an effective feeding mode in still or slowly moving water. Slow beat serves mainly as a respiratory tool (Anderson, 1994). Detection of the extension mode, which occurs naturally in moderate water flow, was observed only through personal watching.

The results showed that the cirral activities switched between fast, normal, and rest phases in variable durations. Gwilliam (1987) stated that two functional types of motor neuron exist, fast and slow, both terminating at the cirri base. The data recorded fast beats of up to 100 beats/min. Rates of up to 150 beats per mintue may by achieved during fast beat in *Semibalanus balanoide (*Barber and Trefry, 1981).

High concentrations of heavy metals are toxic to living organisms (Irwin *et al.,* 1997). Cirral activity is clearly inhibited under exposure to heavy metals in all concentrations. Exposure to excess copper causes a reduction in the rate of movement of the feeding appendages and the feeding activity (cirral beat). Disturbances in the *B. eburneus* cirral rhythms, followed by their disappearance, were observed both under 24 hr and 96 hr LC_{50} copper exposure. Powell and White (1989) reported that in two species of barnacles, exposure to excess copper (80 µg/L or more) causes a reduction in the rate of movement of the feeding appendages.

The adaptive advantage of such an endogenous rhythm would be the ability to anticipate periods of activity and inactivity of food availability. Daily vertical migrations and feeding rhythms of zooplankton influence the diet composition (Hattori and Saito, 1997). Moreover, diurnal, tidal, and seasonal rhythms related to feeding and reproduction are features of shallow-water barnacle life (Anderson, 1994). But much remains to be learned on how variations in respiratory and cirral rhythms are initiated and controlled.

In conclusion, behavioural changes are believed to be a sensitive indicator of exposure to toxicants but can be very difficult to quantify. The results from the present work have shown that adult barnacles are potentially ideal monitors of pollution, particularly of copper. Furthermore, monitoring of these cirral activity patterns could be extended to investigate different environmental interactions of the barnacles under controlled experimental conditions. The introduction of computer-aided monitoring systems, however, supports the acquisition of physiological and/or behavioural data in the laboratory over long periods.

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النشاط المتكرر للبرنقيلات كمؤشر سلوكى حيوى للدلالة على التلوث بالنحاس فى بحيرة التمساح ، قناة السويس ، مصر

علاء الدين عبد العزيز سلام ، ماهى عبد الفتاح غباشى ، الهام علي الخواص ، عبد الفتاح على غباشى
قسم علم الحيوان ، كلية العلوم ، جامعة قناة السويس ، الإسماعيلية

الملخص العربـــى

يهدف هذا البحث إلى تقديم حيوان البرنقيلات الجليسة كمؤشر سلوكى حيوى فى بحيرة التمساح ، قناة السويس ، مصر. و قد تم بنجاح إستخدام تصميم خاص للتسجيل الالى لحركة الذؤابات فى البرنقيلات ، و لقد أجريت هذه التجارب على نوعين من البرنقيلات هما "بالانس إيبيورنس" *Balanus eburneus* ، و "بالانس أمفترايت" *B. amphitrite* .

إن الحركة النموذجية للذؤابات تهدف بصفة أساسية للحصول على الغذاء فى هذا الحيوان . لوحظ فى الظروف الطبيعية أن معدل ضربات هذه الذؤابات يتأرجح بين معدل سريع يبلغ حوالى 100 ضربة فى الدقيقة ، و معدل آخر عادى يصل إلى حوالى 50 ضربة فى الدقيقة. و قد أظهر البرنقيل "بالانس إيبيورنس" نشاطاً متكرراً لهذه الذؤابات فى الظروف المعملية الثابتة من حيث الإظلام التام و كذا درجة الحرارة حوالى 28^{o}م ، حيث كان نشاط هذه الذؤابات يتكرر كل حوالى 12 ساعة ، أى يتزامن مع توقيت حركة المد و الجزر فى بيئته الطبيعية.

و من ناحية أخرى فقد تم ملاحظة نشاط الذؤابات تحت تركيزات مختلفة من النحاس ، و لتعيين التركيزنصف المميت LC_{50} فقد تم تعريض الحيوانات إلى تركيزات مختلفة كالتالى: 0.2،0.4،0.6،0.8،1،1.5،2،2.5،3،3.5 ملليجرام لكل لتر و ذلك لتعيين درجة التركيز التى تميت نصف الحيوانات بعد 24، 48، 72، 96 ساعة ، و قد وجد أنها بلغت 1.33،0.635،0.40،0.291 ملليجرام لكل لتر فى حالة البرنقيل "بالانس إيبيورنس" ، بينما كانت 1.184،0.493،0.272، 0.139 ملليجرام لكل لتر فى حالة البرنقيل "بالانس أمفترايت" على التوالى. أظهرت النتائج أن نشاط الذؤابات قد تأثر تأثراً طردياً مع التركيز نصف المميت بعد 24 ، 96 ساعة من التعرض للنحاس فى ماء البحر.

و خلصت هذه الدراسة إلى أنه يمكن اعتبار النشاط المتكرر للذؤابات و هو سلوك أساسى للبرنقيلات كمؤشر جيد لمتابعة درجة التلوث فى بحيرة التمساح، ومن ناحية أخرى فإن التصميم المستخدم فى هذه الدراسة يمكن أن يعمم لدراسة التفاعلات العديدة للبرنقيلات مع البيئة المحيطة.