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**PHENOMENON OF THE COMPLETE SUPPRESSION OF CARDIAC ACTIVITY
IN THE BLACK SEA SCORPIONFISH *SCORPAENA PORCUS* (SCORPAENIDAE)
DURING AN ALERTNESS REACTION**

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Teleosts serve as experimental models for the physiological and pathophysiological processes investigation, in particular those related to the heart work. Methods allowing to analyze the frequency parameters of the heart functioning over a long period of time require taking into account the peculiarities of fish behavioral reactions, that can affect the results of the experiment. The aim of this study was to examine the effect of the simplest test loading (sound stimulus) on the frequency parameters of the heart activity recorded by the fiber-optic method. The objects of the study were adults of *Scorpaena porcus* (12–15 cm long, 80–120 g in weight). In the course of experiments, each scorpionfish was kept in a separate aquarium with seawater (400×400×350 mm), with constant temperature (21 ± 0.5 °C and given oxygen content (5.5–6.7 mg·L⁻¹, normoxia). The heart rate (HR) recording was carried out by an invasive fiber-optic method, the essence of which is to transmit the radiation of the infrared semiconductor laser of the photoplethysmograph through a thin fiber-optic cable to the pericardial membrane of the heart and then to fix the signal reflected from the contracting myocardium in the photodetector. During implantation of the photoplethysmograph light guides, the fish were anesthetized by placing them in an anesthetic solution (urethane, 2.4 g·L⁻¹ of seawater). In the fornix of the opercular cavity above the area of the conditional heart projection, a minimal dissection of the lining epithelium was performed, through which the underlying tissues were sequentially separated by a blunt method until the pericardial membrane was reached without breaking it. Through the lumen formed in the tissues, two optical light guide sensors were introduced to the surface of the pericardial membrane. Further, free-swimming scorpionfish participated in the experiment after a day of rehabilitation after the surgery. Additionally, we assessed the functional state of the animals by visual fixation of respiratory activity by the quantity of movements of the opercular covers *per* minute. During studying the test loading effect on the correct registration of the scorpionfish HR, the phenomenon of temporary complete suppression of cardiac activity was revealed, which manifested itself upon presentation of sound stimuli (alertness, “freezing” reaction). The duration of cardiac arrest was 31 to 50 seconds; it was accompanied by the cessation of movement of the opercular covers (respiratory arrest, apnea). During the restoration of cardiac activity, two types of physiological reactions were noted. The first type of recovery reaction was characterized by a simultaneous 1.5-fold increase in the HR and a 2-fold enhancement in the photoplethysmograph signal amplitude. The second type of reaction was accompanied by a rise in the HR by 22 % ($p < 0.05$) against the backdrop of a decrement in the signal amplitude of the photoplethysmograph sensors by 28 % ($p < 0.05$); within ~ 120 seconds, the scorpionfish HR returned to baseline. It is assumed that the short-term delay in the scorpionfish cardiac activity is based on the phenomenon of cardiorespiratory coupling and synchronization. The behavioral reaction in the form of suppression for the cardiac and simultaneously respiratory activity generation ensures the complete absence of acoustic and electrical signals, which unmask an ambush predator location, and contributes to the scorpionfish survival.

Keywords: fish, *Scorpaena porcus*, heart, cardiorespiratory coupling, alertness reaction

Teleosts largely retain the similarity of some anatomical and physiological features with mammals, which makes them a convenient and reliable experimental model for studying fundamental / “conserved” physiological and pathophysiological processes occurring in different classes of animals (Gut et al., 2017). The two-chambered fish heart is similar to the human heart in the peculiarities of its development, regeneration, electrical properties of the myocardium, and the presence of several pathological syndromes, in particular long QT syndrome (Sun et al., 2009). There are various approaches to assessing the cardiac activity of teleost fish, which imply the use of variety of instrumental “tools” ensuring the solution of specific experimental problems. In any case, a method allowing to analyze the rate characteristics of the heart rhythm over a long period of time (up to several days) requires taking into account the features of fish behavioral reactions, that can affect the results of the experiment. As an object of research, we chose the Black Sea scorpionfish (sea ruff) *Scorpaena porcus*, which is resistant to a number of stress factors (Kolesnikova & Golovina, 2020 ; Soldatov et al., 2020).

The aim of this study is to examine the effect of the simplest test loading (sound stimulus) on the frequency parameters of the scorpionfish heart activity recorded by the fiber-optic method. The task of the work was to investigate the influence of random environmental factors on the results of recording the scorpionfish heart rate.

MATERIAL AND METHODS

The object of the study was 7 adult specimens of *Scorpaena porcus* Linnaeus, 1758, 12–15 cm long, 80–120 g in weight. The fish were caught in August – September with a fixed seine in Sevastopol water area and delivered to the laboratory in 60-L plastic tanks, with aeration. To relieve stress after transportation, the fish were kept for 7 days in a flow-through aquarium; only actively moving and feeding individuals were used. In the course of experiments, each scorpionfish was kept in a separate aquarium-stand with seawater (400×400×350 mm) under controlled temperature (21 ± 0.5) °C and oxygen content of 5.5–6.7 mg·L⁻¹, normoxia (Soldatov et al., 2020).

The recording of the heart rate (hereinafter HR) was performed by the invasive fiber-optic method for the first time on a representative of teleost fish; previously, a similar method was used on crustaceans (Sladkova et al., 2016). The essence of the fiber-optic method is to transmit the radiation of an infrared semiconductor laser of a photoplethysmograph (LVOF-3, manufactured by Research Innovation Center “EcoContour”, Russia) through a thin fiber-optic cable to the pericardial membrane of the heart (Fig. 1A) and then to fix the signal reflected from the contracting myocardium in the photodetector.

After appropriate amplification, filtration, and digitalization, the cardiac signal was analyzed on a computer and recorded in the form of a photoplethysmogram (Fig. 1B), which allows estimating the dynamics of the HR. The fiber-optic method makes it possible to examine the functional state of fish for a long time without affecting their behavior or causing stress. During implantation of photoplethysmograph light guides, the fish adapted to laboratory conditions were anesthetized by placing them in an anesthetic solution (urethane, 2.4 g·L⁻¹ of seawater) (Soldatov, 2005). Then, in the fornix of the opercular cavity above the area of the heart conditional projection, a minimal dissection of the lining epithelium was performed, through which the underlying tissues were sequentially disunited so as to avoid damaging them, until the pericardial membrane was reached without breaking it. Through the lumen formed in the tissues, two optical sensors of light guides were introduced to the surface of the pericardial membrane.

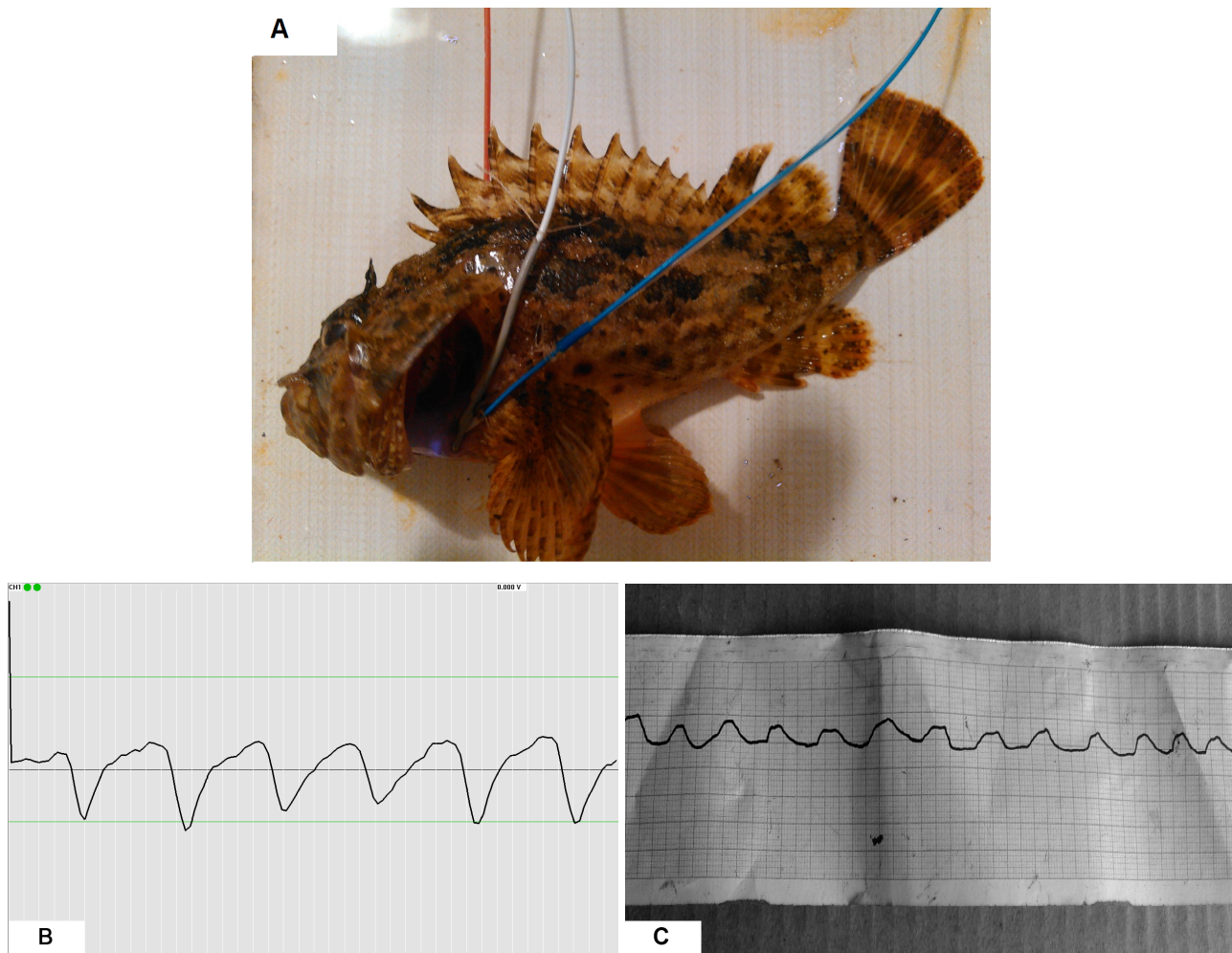


Fig. 1. *Scorpaena porcus* with attached recording sensors (A): 1 and 2 – electrodes of a portable electrocardiograph; 3 and 4 – light guides of photoplethysmograph optical sensors. Steady state cardiac activity of *Scorpaena porcus*: photoplethysmograph registration (B); portable electrocardiograph registration (C)

A stable position of the sensors was ensured by hemming the cables of light guides with silk thread to the skin above the cleithrum and in the dorsal fin area, which made it possible to fix the cables in the vertical position, perpendicular to the longitudinal axis of the scorpionfish body. Later, free-swimming scorpionfish participated in the experiment after a day of rehabilitation after the surgery. Fish reactions were taken into account only to the first two presentations of the stimulus because of partial extinction of subsequent reactions to the stimulus in the form of shortening the time for the manifestation of alertness not canceling the established physiological phenomenon. Photoplethysmograms were compared with ECG (Fig. 1B) obtained using a portable electrocardiograph (EK1T-03M); during ECG recording, modified electrodes were placed at the base of the dorsal and left pectoral fins of the scorpionfish (Fig. 1A). Additionally, the functional state of the animals was assessed by visual fixation of respiratory activity by the number of movements of the opercular covers *per* minute. To study the effect of test loadings on cardiac activity, we chose the simplest sound stimulus: tapping on the aquarium glass, which could affect the physiological reactions of the scorpionfish.

Statistical comparisons were made on the basis of a two-tailed Student's *t*-test; the results are presented as ($M \pm m$).

RESULTS AND DISCUSSION

The obtained photoplethysmograms allowed determining the mean values of the scorpionfish HR at rest, (11.22 ± 1.07) beats·min⁻¹, which corresponded to a similar number of respiratory movements (Fig. 2). When studying the effect of the test loading on the recorded parameters of the myocardium, we revealed the phenomenon of complete suppression of cardiac activity. Thus, a simple tapping on the aquarium glass, along with a hand wave, was accompanied by the emergence of an alertness reaction (“freezing”) in the fish followed by almost complete disappearance of heart contractions (Fig. 2A).

It should be noted that the alertness reaction is related to the cessation of movement of the opercular covers (respiratory arrest, apnea) as well. Several tens of seconds (31 to 50 s; 39.0 ± 7.6 s) after the action of the sound stimulus, the contraction of the scorpionfish heart resumed (Fig. 2B). During the restoration of cardiac activity, two types of physiological reactions were revealed. The first type of recovery reaction ($n = 2$) was characterized by a simultaneous 1.5-fold increase in the HR (tachycardia) and a 2-fold increase in the photoplethysmograph signal amplitude (Fig. 2C). The second type of recovery reaction ($n = 5$) was accompanied by an increase in the HR by 22 % ($p < 0.05$) against the backdrop of a decrease in the signal amplitude of the photoplethysmograph sensors by 28 % ($p < 0.05$) (Fig. 2D). Within approximately 120 s, the scorpionfish HR returned to baseline. Such a reaction of the cardiac muscle of teleost fish has not been previously described in the literature.

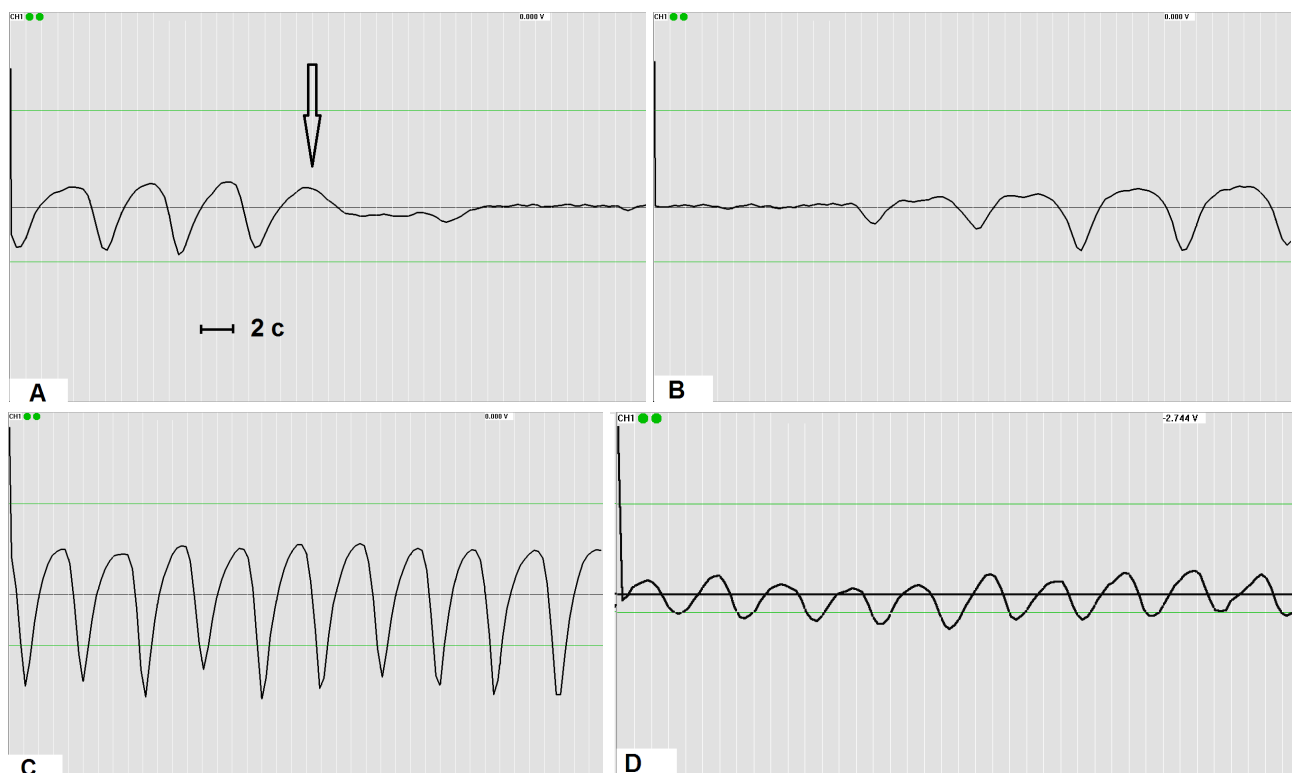


Fig. 2. Photoplethysmograms of the scorpionfish *Scorpaena porcus*: A – delayed cardiac activity of the scorpionfish during the alertness reaction under the tapping on the aquarium wall (the moment of stimulus presentation is marked by an arrow); B – restoration of the scorpionfish cardiac activity after the stimulus and the cancellation of alertness reaction; C – an increase in the heart rate and its amplitude in the period following the cancellation of the alertness reaction (first type of restorative physiological reaction); D – an increase in the heart rate at low amplitude in the period following the cancellation of the alertness reaction (second type of restorative physiological reaction)

As known, the contractions of the heart of vertebrates are initiated by a built-in myogenic generator of heart rhythm; in addition, the steady state cardiac activity values can be regulated by the mechanisms of the central nervous system, which, using the efferent pathways of the autonomic nervous system, have both inhibitory parasympathetic and excitatory sympathetic effects on the heart work (Taylor et al., 2014).

There is a close temporal relationship between cardiac contractions and respiratory activity of fish in the form of cardiorespiratory coupling (hereinafter CRC) and cardiorespiratory synchronization (hereinafter CRS) reaching a ratio of 1:1 (Satchell, 1959 ; Taylor, 1992 ; Taylor et al., 2009); those are determined by the specificity of water flow movement through the branchial apparatus and its perfusion with circulating blood. This close coordination between ventilation and perfusion proportions helps in optimizing gas exchange related to circulatory counterflow in the branchial tissue (Taylor, 1992). CRS appears to depend on a combination of “direct” central and reflex, associated with different receptors, control mechanisms (Taylor, 1992 ; Taylor et al., 1999). The anatomical basis for the CRS manifestation is the close proximity of cardio and respiratory centers in the brainstem, including elements of the motor nuclei of trigeminal (V), facial (VII), glossopharyngeal (IX), and vagal (X) cranial nerves (hereinafter CNs); motor nuclei of CNs are connected with each other and with the reticular formation.

Certain CRS elements are also present in mammals in the form of an HR increase during inspiration defined as respiratory sinus arrhythmia (RSA) (Jordan & Spyer, 1987). The cause for RSA is considered to be respiratory-related fluctuations of the inhibitory action of the vagal nerve (*n. vagus*, X CN) on myocardial contractile activity, which are formed under the depressor effect of the inspiratory neurons of the ventral respiratory group localized in the double nucleus (*nucleus ambiguus*) on *n. vagus* preganglionic neurons. In teleost fish, “varying” HR includes “respiratory components” as well. Activity of *n. vagus* can provide the predominant mode of regulation of the fish cardiac function (Taylor et al., 1999), which is eliminated by vagotomy (*n. vagus* transection) (Cambell et al., 2004). As believed, *n. vagus* inhibitory effect is realized through m-cholinergic receptors directly related to the myogenic mechanism of the HR generation (Taylor et al., 1999).

In marine fish, the tonic activity of *n. vagus* cardiac branches is regulated relatively to the influx of afferent impulses from the chemo- (Cambell et al., 2004) and mechanoreceptors of the branchial apparatus (Young et al., 1993). Thus, in an experiment with stimulation of *n. vagus* gill branches, the presence of afferent feedback was established, which probably “originates” from the branchial mechanoreceptors and controls the rhythmic activity of *n. vagus* cardiac branches (Young et al., 1993); moreover, individual *n. vagus* preganglionic neurons show a distinct response to mechanical stimulation of the branchial septa in the form of such a surge in their activity, as a result of which transient bradycardia may develop (Barrett & Taylor, 1985). Pronounced suppression of the HR during stimulation of *n. vagus* is shown in European plaice *Pleuronectes platessa* (Cobb & Santer, 1972) and common carp *Cyprinus carpio* (Saito, 1973). Moreover, it was established that an increase in the HR, that develops after vagotomy, was also accompanied by the cancellation of the so-called approach reflex with the characteristic periods of bradycardia observed during alert in intact rainbow trout *Oncorhynchus mykiss* (Priede, 1974).

As mentioned above, in the scorpionfish, suppression of cardiac muscle contractions was observed simultaneously with the cessation of movements of the opercular covers (respiratory arrest). Fish retain the features of a more primitive metameric organization of the respiratory system compared to mammals (Satchell, 1959), when numerous gill mechanoreceptors are provided with inhibitory afferent fibers in the gill branches of VII, IX CNs, including *n. vagus* (Sutterlin & Saunders, 1969). The afferent impulses input initiated during inspiration from the gill mechanoreceptors is carried by *n. vagus* fibers

and is involved in the regulation of gill respiration in fish (Satchell, 1959); probably, the mentioned mechanism in a certain approximation is analogous to the Hering – Breuer reflex of mammals, since in fish, in addition to episodes of bradycardia, stimulation of *n. vagus* is accompanied by breath holding (Satchell, 1959). At the same time, in mammals, breath holding in the form of transient apnea occurs during pharmacological blockade of *n. vagus* (Harris & Milsom, 2001). The question of whether it is possible in fish to situationally eliminate/modify afferent input of *n. vagus* before the development of apnea (Harris & Milsom, 2001) or use the feedback between the reflex cessation of breathing and the activity of *n. vagus*, which mediates cardiac suppression, requires further study.

The hindbrain containing pons and cerebellum is also involved in the formation of respiratory movements in fish (Ballintijn & Roberts, 1982). This conclusion is based on the results of transection of the fish rhomboid brain along the caudal and rostral borders, which was not accompanied by the cancellation of the respiratory activity provided by the somatic muscles of the branchial apparatus. The participation of the coordinating motor center (*inter alia* as a universal regulator of somatic and autonomic functions) in the mechanisms of respiratory activity generation of fish can facilitate the reflex cessation of the branchial apparatus ventilatory movements, which are indirectly associated with the mechanisms of the HR formation.

It can be assumed as follows: the main trigger of the “conservative” CRS mechanism is respiratory modulation of heart contractions (Taylor et al., 2014), which involves *n. vagus* “pathways”. The complete suppression of cardiac activity, that we established during the alertness reaction of the scorpionfish, is a special case of CRS, since the cessation of rhythmic contractions of the heart muscle was noted simultaneously with the termination of movements of the opercular covers providing the respiratory process. Obviously, in the scorpionfish body, CRS degree acquires such a level, at which the reflex cessation of respiratory movements causes a complete suppression of the heart work.

It is known that fish life takes place in a variety of physical fields (light, acoustic, and electric ones) (Barinova & Asylbekova, 2019). At the same time, the visibility range of objects in water is very small and fluctuates within bounded limits (one to two tens of meters). In a dense aquatic environment, mechanical, sound vibrations spread very quickly over considerable distances, which can either contribute to unmasking the animals, or warn in advance about the approach of a potential danger (Protasov, 1965). In addition to acoustic signals (*inter alia* the sounds of working covers of the branchial apparatus, contractions of the heart chambers, crunching of the skeletal joints, and hydrodynamic sounds), very weak electrical discharges resulting from muscle contraction emanate from almost all marine animals (Protasov, 1972), and this attracts predatory fish capable of detecting the electric fields of other living things. Scorpionfish is a recognized ambush predator, for which it is important not to reveal its presence when a potential prey approaches. Apparently, a similar behavioral reaction of the scorpionfish in the form of complete “freezing” should also be manifested in the case when it itself is as a potential prey for larger predators. In both situations, the interruption of generation and the complete absence of acoustic and electrical signals indicating the location of the disguised scorpionfish contribute to its survival as a species. Obviously, the phenomenon we established corresponds to the maxim that physiological transformation presupposes an emphasis on the role of the organism own activity in the evolutionary process, highlighting that “to live means to react, and by no means to be a victim” (Chaikovskii, 1990).

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ФЕНОМЕН ПОЛНОГО ПОДАВЛЕНИЯ СЕРДЕЧНОЙ ДЕЯТЕЛЬНОСТИ ЧЕРНОМОРСКОЙ СКОРПЕНЫ *SCORPAENA PORCUS* (SCORPAENIDAE) ПРИ РЕАКЦИИ НАСТОРОЖЕННОСТИ

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Костистые рыбы известны как экспериментальные модели для изучения физиологических и патофизиологических процессов, в частности связанных с работой сердца. Методы, позволяющие производить анализ частотных характеристик сердечного ритма в течение длительного периода времени, нуждаются в учёте особенностей поведенческих реакций рыб, способных повлиять на результаты эксперимента. Целью работы было изучить воздействие простейшей тестовой нагрузки (звуковой раздражитель) на частотные параметры сердечной деятельности, фиксируемые волоконно-оптическим методом. Объект исследования — взрослые особи *Scorpaena porcus* длиной 12–15 см, массой 80–120 г. В ходе экспериментов каждую скорпену содержали в отдельном аквариуме с морской водой размером 400×400×350 мм с постоянной температурой (21 ± 0,5) °C и регулируемым содержанием кислорода (5,5–6,7 мг·л⁻¹, нормоксия). Регистрацию частоты сердечных сокращений (ЧСС) производили инвазивным волоконно-оптическим методом, суть которого состоит в передаче излучения инфракрасного полупроводникового лазера фотоплетизмографа по тонкому волоконно-оптическому кабелю к перикардиальной мембране сердца и в последующей фиксации отражённого от сокращающегося миокарда сигнала в фотоприёмнике. При имплантации световодов фотоплетизмографа рыбу наркотизировали путём помещения в раствор анестетика (уретан, 2,4 г·л⁻¹ морской воды). В своде оперкулярной полости над областью условной проекции сердца производили минимальное рассечение выступающего эпителия, через которое подлежащие ткани последовательно разъединяли тупым методом до достижения перикардиальной мембраны, не прорывая её. Через образовавшийся в тканях

просвет к поверхности перикардиальной мембраны вводили два датчика световодов. В дальнейшем свободно плавающие скорпены принимали участие в эксперименте спустя одни сутки после хирургического вмешательства. Дополнительно нами было оценено функциональное состояние животных путём визуальной фиксации дыхательной активности по количеству движений оперкулярных крышек в минуту. При изучении влияния тестовых нагрузок на корректность регистрации ЧСС у скорпены был выявлен феномен кратковременного полного подавления сердечной деятельности, проявлявшийся при предъявлении звуковых стимулов (реакция настороженности, «замирание»). Длительность остановки сердечных сокращений составляла 31–50 с., она сопровождалась прекращением движения оперкулярных крышек (остановка дыхания, апноэ). При восстановлении сердечной деятельности отмечали два типа физиологических реакций. Для восстановительной реакции первого типа характерно одновременное увеличение ЧСС в 1,5 раза и амплитуды сигнала фотоплетизмографа в 2 раза. Второй тип восстановительной реакции сопровождался увеличением ЧСС на 22 % ($p < 0,05$) на фоне снижения амплитуды сигнала датчиков фотоплетизмографа на 28 % ($p < 0,05$); в пределах ~ 120 с. ЧСС скорпены возвращалась к исходным показателям. Предполагается, что в основе кратковременной задержки сердечной деятельности скорпены лежит явление кардиореспираторного сопряжения и синхронизации. Поведенческая реакция в виде подавления генерации сердечной и одновременно дыхательной активности обеспечивает отсутствие акустических и электрических сигналов, демаскирующих местоположение хищника-засадчика, и способствует выживанию скорпен.

Ключевые слова: рыбы, *Scorpaena porcus*, сердце, кардиореспираторное сопряжение, реакция настороженности