

## The Anaesthetics Effects of Quinaldine Sulphate and/or Diazepam on Sea Bass (*Dicentrarchus labrax*) Juveniles

Mahmut YANAR, Metin KUMLU

Faculty of Fisheries, Çukurova University, 01330 Balcalı, Adana - TURKEY

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**Abstract:** In this study, the effects of an anaesthetic quinaldine sulphate (QS) and a muscle relaxant, diazepam (D), on sea bass (*Dicentrarchus labrax*) juveniles (8-9 g) were investigated. When used together with D, the anaesthetic effect of QS was significantly increased. The fish entered light anaesthesia at 15 ppm QS, as compared to 5 ppm QS + 1 ppm diazepam (D). Similarly, a deep anaesthesia level was reached at only 7.5 ppm QS + 1 ppm D as compared to 20 ppm of QS. The use of QS alone at high concentrations (7.5-25 ppm) increased mortality from 10% to 100%. No post-exposure mortality occurred in any of the fish treated with QS plus D at all anaesthesia levels. Depending on the anaesthetic concentrations used, the time to loss of equilibrium and recovery time were 1-3 and 2-6 min, respectively. The combination of QS and diazepam significantly decreased the excitement and hyperactivity of the fish in confined space without leading to mortality. It appears that the suitable light and deep sedation stages of anaesthesia for transportation and handling of the sea bass juveniles (8-9 g) were obtained with dosages of 5 ppm QS + 2 ppm D and 7-10 ppm QS plus 2 ppm D, respectively.

**Key Words:** Sea bass, *Dicentrarchus labrax*, quinaldine sulphate, diazepam, anaesthesia

### Kinaldin Sülfat ve/veya Diazepam'ın Levrek (*Dicentrarchus labrax*) Yavruları Üzerindeki Anestezik Etkileri

**Özet:** Bu çalışmada, anestezi bir madde olan kinaldin sülfat (QS) ve bir kas gevşetici olan diazepam (D)'in, levrek (*Dicentrarchus labrax*) yavruları (8-9 g) üzerindeki anestezik etkileri araştırılmıştır. D kullanımı, QS'in balıklar üzerindeki anestezik etki gücünü artmıştır. Balıklar hafif anesteziyeye 15 ppm QS konsantrasyonu ile girerken, aynı anestezisi seviyesine 5 ppm QS + 1 ppm diazepam (D) konsantrasyonu ile ulaşılmıştır. Benzer şekilde, balıklar derin anesteziyeye 20 ppm QS konsantrasyonu ile girerken, aynı anestezisi seviyesine 7.5 ppm QS + 1 ppm D konsantrasyonu ile girilmiştir. QS'in yüksek konsantrasyonlarda tek başına kullanılması (7.5-25 ppm) mortaliteyi %10'dan %100'e çıkartırken, QS'in D ile kombine kullanılması durumunda hiç bir anestezisi seviyesinde mortalite görülmemiştir. Kullanılan anestezik madde konsantrasyonlarına bağlı olarak, anesteziyeye girme ve anesteziden çıkma süreleri sırasıyla 1-3 ve 2-6 dakika arasında gerçekleşmiştir. QS ile D'in birlikte kullanımı, balıklarda ölümleri ortadan kaldırmış ve ekstasyon ile hiperaktiviteyi de önemli ölçüde azaltmıştır. Levrek yavrularının elle muamele ve taşınmaları esnasında hafif ve derin anestezisi seviyeleri için en uygun konsantrasyonların sırasıyla 5 ppm QS + 2 ppm D ve 7-10 ppm QS + 2 ppm D olduğu saptanmıştır.

**Anahtar Sözcükler:** Deniz levreği, *Dicentrarchus labrax*, kinaldin sülfat, diazepam, Anestezisi

### Introduction

Anaesthetics have long been used in the handling and transportation of fish to reduce the mortality which occurs as a result of excitement and hyperactivity in confined areas (1). Many chemicals, e.g., quinaldine (2), quinaldine sulphate (3,4,5,6), MS-222 (7,8,9), propanidid (10), benzocaine-hydrochloride (11), metomidate, chlorobutanol and phenoxyethanol (12, 9), have been used as anaesthetics for fish. Quinaldine is inexpensive, and effective at very low concentrations and has a low toxicity and short fish recovery time (13). However, poor solubility in water, long induction time and strong odour are the drawbacks of this anaesthetic.

Quinaldine sulphate eliminates the water solubility and odour problems and reduces the induction time (14). At present, quinaldine sulphate is one of the most commonly used anaesthetics in aquaculture. Although quinaldine produces a total loss of equilibrium at deep anaesthesia level, the fish do not completely lose reflex responses (15). This is undesirable during handling and particularly in the case of surgical procedures of fish (16).

It is suggested that the use of quinaldine together with triacin overcomes the reflex twitching problem (17,18). It is known that muscle relaxants reduce excitation, hyperactivity, respiration rate and rigidity of the muscles. Intramuscular injection of muscle relaxants

(e.g., gallamine triethiodide, tubocurarine chloride and pancuronium bromide) has been used to eliminate the reflex problem encountered in anaesthetised fish by quinaldine (14). Diazepam, which is one of the diazepines, is frequently used to decrease muscle rigidity and excitation in combination with appropriate anaesthetics in man (19).

The purpose of this study was to investigate the effects of the anaesthetic quinaldine sulphate alone and in combination with a muscle relaxant (diazepam) on European sea bass (*Dicentrarchus labrax*) juveniles.

## Materials and Methods

*Dicentrarchus labrax* juveniles (8-9 g) were supplied by a local fish farm (Akuvatur Fish Farm, Adana, Turkey). The experiment was carried out in the laboratory of the Marine Experimental Station, Faculty of Fisheries, Çukurova University, Adana, Turkey.

Prior to starting the experiment, the fish were acclimatised to experimental conditions for two weeks in two 10-ton round fibreglass tanks. The experiment was performed in 3-L flat bottom glass flasks in two replicates. The tests were conducted at 24-25°C and 38 ppt salinity. The pH and dissolved oxygen were maintained at 7.9-8.2 and 7-8 mg L<sup>-1</sup>, respectively.

Quinaldine sulphate (QS) was distributed into the flasks at concentrations of 2.5, 5.0, 7.5, 10, 15, 20 and 25 ppm. In addition to QS, diazepam (Deva Company, İstanbul, Turkey) obtained from a local pharmacy in 10 mg ampoules was also added into some of the flasks at concentrations of 0.5, 1.0 and 2 ppm (see Table). After stirring the water of the flask with a glass rod for better dispersal of the anaesthetic, 5 fish which had been starved for 48h were stocked into each of the flasks. Continuous aeration was supplied through airstones attached to a plastic tube. The response of each individual fish in each test media was immediately recorded from the stocking until the end of the experiment.

The exact time taken for the fish to partially or completely lose equilibrium was noted. The fish were observed for 1, 5, 10 and 20 h after the stocking. Following these periods, any fish that lost its equilibrium was transferred to a 3-L glass flask filled with anaesthetic free water to record recovery time. The fish that recovered from the anaesthesia were also observed for another 48 h to observe the post-exposure effect of the

treatments. The fish were not fed either during or after the experimental period.

## Results

The time for the fish to enter the desired anaesthesia level (induction time) ranged between 0.5 and 3 min depending on concentration of the anaesthetic used. Increase in the concentration of QS decreased the time of entrance to anaesthesia. Recovery time from anaesthesia, which ranged between 2 and 6 min, however, increased with the increasing concentration of the anaesthetic (Table). Induction time was 1-2 min for light sedation and 0-0.5 min for deep sedation. Recovery times from light sedation and deep sedation were 3-5 and 4-6 min, respectively.

Four different sedation levels were identified in the fish treated with the anaesthetics:

### \* tranquillity period:

- slow swimming
- slight increase in opercular rate

### \*\* Excitation period:

- unrest
- voluntary swimming still possible
- increase in opercular rate
- high reaction to external stimuli

### \*\*\* Light anaesthesia level:

- slow turning to one side
- still reaction to external stimuli particularly in the fish treated with QS alone
- high opercular rate
- loss of co-ordination
- excrement discharge

### \*\*\*\* Deep anaesthesia:

- lying on one side without movement
- opercular movement very high (up to 200 min<sup>-1</sup>) in some of the fish and very low (9-17 min<sup>-1</sup>) in others
- increase in excrement discharge
- high reaction to external stimuli in fish with a high opercular rate, and no reactions to external stimuli in those with a slow opercular rate

When diazepam (D) was used together with quinaldine sulphate (QS), the fish entered anaesthesia at lower concentrations than when only QS was used. The tranquillity period (\*) was reached at 5 ppm with QS and at 2.5 ppm with QS plus 0.5 ppm D. While the fish attained excitation period (\*\*) at 7.5 ppm with QS, this level is reached at 5 ppm QS + 0.5 ppm D. The fish entered light anaesthesia (\*\*\*) at 15 ppm QS compared to 5 ppm QS + 1 ppm D. Similarly, deep anaesthesia level was reached at only 7.5 ppm QS + 1 ppm D as compared to 20 ppm of QS. D alone did not produce anaesthesia in fish (Table).

QS alone was not suitable for the light or deep anaesthesia of sea bass juveniles. Even at light anaesthesia level (at 15 ppm), 10% of the fish died following stocking. At deep anaesthesia level, which was reached at

20-25 ppm, mortality ranged from 30 to 100%. No fish mortality occurred during the post-exposure period.

The opercular rate at the same anaesthesia levels with QS was much higher than that with the combination of QS + D. The opercular rates of the fish at light anaesthesia with QS at 15 ppm and with QS + D (7.5 ppm QS + 0.5 ppm D or 7.5 ppm QS + 2 ppm D) were 205 min<sup>-1</sup> and 132-166 min<sup>-1</sup>, respectively. An increase in QS concentration from 2.5 to 15 increased the opercular rate from 130 to 205 min<sup>-1</sup> (Table). At deep anaesthesia, the opercular rate of fish subjected to 20-25 ppm QS decreased to 9-17 min<sup>-1</sup>. However, 30 to 100% of the fish did not recover from this anaesthesia level. In contrast, the fish brought to deep anaesthesia with QS plus D had an opercular rate of 138 min<sup>-1</sup>, and 100% of the fish successfully recovered from this anaesthesia level.

Table. The effects of quinaldine sulphate (QS) and quinaldine sulphate + diazepam (QS + D) on sea bass juveniles. - refers to 'none'. N was 2 for each treatment, and in each n five fish were observed.

Anaesthetic concentration (ppm)	Induction time (min)	Recovery time (min)	Anaesthesia level	Opercular rate (min <sup>-1</sup> )	Experimental mortality (dead fish/total no of fish)				Post-exposure mortality 48 h
					1 h	5 h	10 h	20 h	
2.5 QS	-	-	-	130	-	-	-	-	-
2.5 QS + 0.5 D	1-3	2-3	*	125	-	-	-	-	-
2.5 QS + 1.0 D	1-3	2-3	*	125	-	-	-	-	-
2.5 QS + 2.0 D	1-3	2-3	*	121	-	-	-	-	-
5 QS	1-3	2-3	*	155	-	-	-	-	-
5 QS + 0.5 D	1-3	3-5	**	156	-	-	-	-	-
5 QS + 1.0 D	1-2	3-5	***	142	-	-	-	-	-
5 QS + 2.0 D	1-2	3-5	***	132	-	-	-	-	-
7.5 QS	1-2	3-5	**	174	-	-	1/10	-	-
7.5 QS + 0.5 D	1-2	3-5	***	166	-	-	-	-	-
7.5 QS + 1.0 D	1-2	4-6	****	163	-	-	-	-	-
7.5 QS + 2.0 D	1-2	4-6	****	138	-	-	-	-	-
10. QS	1-2	3-5	**	193	-	-	-	-	-
10 QS + 0.5 D	0-0.5	4-6	****	180	-	-	-	-	-
10 QS + 1.0 D	0-0.5	4-6	****	176	-	-	-	-	-
10 QS + 2.0 D	0-0.5	4-6	****	176	-	-	-	-	-
15 QS	0-0.5	4-6	***	205	-	-	1/10	-	-
20 QS	0-0.5	4-6	****	17	2/10	-	3/10	-	-
25 QS	0-0.5	-	****	9	10/10	-	-	-	-
2 D	-	-	-	101	-	-	-	-	-
Control	-	-	-	115	-	-	-	-	-

\* : tranquillity period; \*\* : Excitation period; \*\*\* : Light anaesthesia level; \*\*\*\* : Deep anaesthesia

## Discussion

The QS concentrations tested in this study did not induce light or deep anaesthesia without causing mortality in the juveniles of sea bass. From 10 to 30% mortality occurred at light or deep sedation concentrations of this anaesthetic. Even during the excitation period, 10% of the fish died 10 h after the termination of the experiment. The fish were able to enter the light sedation level with QS at 15 ppm, but at this concentration, their opercular rate almost doubled and 10% of them died following the experiment. The fish that entered anaesthesia had a high respiration rate and were still sensitive to external stimuli. This observation agrees with the finding of Schoettger and Steucke (17), who stated that fish exposed to quinaldine retain a strong reflex response to being touched even when they have totally lost their equilibrium. Similar statements have also been made by Schoettger and Julin (20) for trouts and by Schram and Black (16) for grass carp. When the muscle relaxant diazepam was used together with the anaesthetic, the above disadvantages were partially eliminated. The fish mortality and excitation observed in those treated with only quinaldine at high dosages did not occur with quinaldine plus diazepam. For surgery purposes, intramuscular injection of skeletal muscle relaxants such as gallamine triethiodide, tubocurarine chloride or pancuronium bromide has already been used to eliminate the reflex problem in anaesthetised fish (14). Diazepins decrease muscle rigidity and excitation when used in combination with appropriate anaesthetics in man (19). The current study demonstrates that diazepam, as a muscle relaxant, in combination with QS also has similar effects in fish.

It is well known that some anaesthetics may be more suitable for one species than others. The effective concentration of an anaesthetic varies according to sex, age, physiological state of individuals, water temperature and hardness, biomass of fish, induction time and duration of exposure (14). QS is suggested as a good anaesthetic for various fish species. The recommended QS concentration for sedation (reduced reaction to external stimuli without loss of equilibrium) of adult striped sea bass (*Morone saxatilis*) at a water temperature of 23°C was 5 ppm (21). The same author, however, stated that the lowest effective concentration for immobilisation was 25 ppm. The lowest effective concentration of QS for red

drum (*Sciaenops ocellatus*) at 26°C was 35 ppm (4). Guo et al. (5,6) reported that in the transportation of platyfish (*Xiphophorus maculatus*), QS was the second most effective anaesthetic in decreasing the metabolic wastes. In addition, it is known that QS is also successfully used locally, at 3-10 ppm, in adult sea bass (*D. labrax*) during hormone injections for propagation. Despite the above reports, the present study demonstrates that sea bass juveniles (8-9 g) are too sensitive to QS for it to be used alone.

Although QS was able to induce a deep sedation level at 20-25 ppm, the opercular rate of the fish almost ceased and finally from 30 up to 100% of the fish did not recover from the anaesthesia. Lower dosages of QS did not produce the desired sedation level. Hence, the present study has shown that QS is not a suitable anaesthetic for deep or even light anaesthesia for sea bass juveniles under the current experimental conditions. The anaesthesia level is significantly increased when diazepam was used in combination with QS. For example; while sea bass juveniles reached deep sedation level at 7.5 ppm QS plus 1 ppm D, this level of anaesthesia, with only QS, was attained at a much higher concentration of 20 ppm (Table). When diazepam was used in combination with QS, no mortality was recorded at even deep sedation dosages. This indicates that the use of diazepam increases the safety level of the anaesthetic for sea bass juveniles.

Though there is dispute in the literature, it is generally accepted that light anaesthesia is desirable during the transportation of fish (14). Anaesthetised fish at deep sedation levels lose equilibrium, and hence may sink to the bottom, pile up and finally suffocate (22). It appears that a concentration of 5 ppm QS plus 2 ppm diazepam is suitable for light anaesthesia, and may be used for the transportation of sea bass juveniles. The concentration of 7.5 ppm QS plus 2 ppm diazepam which provides deep anaesthesia may be suitable for marking, surgery and handling. The use of diazepam in combination with QS eliminated fish mortality, decreased the respiration rate of the fish and the response to external stimuli considerably in comparison to those treated only with the anaesthetic. Diazepam is sold in local pharmacies in Turkey for about 0.2 US \$ per ampoule (10 mg) for human medication. If it is supplied in bulk, it is expected that it would cost less.

In conclusion, diazepam, when administered with QS at considerably low concentrations, enhances anaesthesia and eliminates the undesirable effects of QS. The combination of QS and diazepam significantly decreased the excitement and hyperactivity of the fish in confined space without leading to mortality.

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