Overzichtsartikel 213

Potential welfare issues of the Siamese fighting fish (*Betta splendens*) at the retailer and in the hobbyist aquarium

Mogelijke welzijnsproblemen bij de Siamese kempvis (Betta splendens) in sierviswinkels en bij de hobbyist

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Betta splendens is an extremely popular ornamental fish among hobby aquarists. It has an interesting behavioral repertoire, particularly where male aggression and territoriality are concerned. The lack of scientific studies investigating optimal housing conditions in combination with the wide variety of commercially available husbandry products, raises questions about the welfare status of these fish in captivity. In this article, an overview of the available literature on the biology of the betta and general considerations of ornamental fish keeping is given, and environment- and animal-related factors with potential impact on the welfare of Betta splendens are examined. Although more research using biological and physiological indicators is needed, the following factors constituting welfare problems have been identified: an aquarium of limited dimensions, prevalence of Mycobacterium spp. infection, aggression to and from conspecifics or other species in the same aquarium and the limited ability to escape, potential for stress due to prolonged visual contact between males in shops and during shows, and the lack of environmental enrichment in the form of sheltering vegetation.

SAMENVATTING

Betta splendens is een zeer populaire siervissoort in de aquaristiek. Ze heeft een interessant diersoortspecifiek gedrag, vooral wat het territoriale gedrag en de agressie van mannelijke dieren betreft. De schaarse wetenschappelijke informatie over de huisvesting in combinatie met de grote verscheidenheid aan huisvestingsmogelijkheden doet vragen rijzen over het welzijn van deze dieren in gevangenschap. In dit artikel wordt een overzicht gegeven van de voor handen zijnde wetenschappelijke literatuur betreffende de biologie en de gebruiken in de aquaristiek, met als doel omgevings- en diergerelateerde factoren in kaart te brengen die mogelijk een impact hebben op het welzijn van Betta splendens. Hoewel meer onderzoek nodig is, waarin gebruik gemaakt wordt van biologische en fysiologische indicatoren, kunnen enkele mogelijke welzijnsproblemen aangeduid worden: te kleine aquariumafmetingen, prevalentie van Mycobacterium spp., agressie van en naar soortgenoten of andere vissoorten in het aquarium en de beperkte mogelijkheid om eraan te ontsnappen, mogelijke stress door visueel contact tussen mannelijke individuen in winkels en tijdens shows en het gebrek aan omgevingsverrijking die een schuilplaats vormt voor deze vissoort.

INTRODUCTION

Ornamental fish keeping is a very popular hobby (Peter and Tan, 1997; Chapman and Fitz-Coy, 1997; Tlusty, 2002; Whittington and Chong, 2007). Each year, over a billion ornamental fish are sold worldwide (Ornamental Aquatic Trade Association, Whittington and Chong, 2007). Despite the variety in fresh

water fish species, certain species are extremely popular amongst hobbyists, including the tropical fish *Betta splendens* (Chapman and Fitz-Coy, 1997). In Thailand, known for its ornamental fish production, *Betta splendens* represents 10% of the annual fish export (Wiwatchaisaet, as cited in Meejui et al. 2005).

In recent years, scientific interest in the welfare of farmed fish produced for food, has increased (Brand-

son, 2007). In contrast to its popularity however, there is very little scientific literature documenting the optimum for various housing factors in ornamental fish keeping. As a consequence, the hobbyist mostly relies on non-scientific manuals, his/her own experience and that of peers. This lack of scientific knowledge is accompanied by a wide variety of aquaria (in many sizes and shapes), technical equipment (consisting of different types of filtration, heating and lighting systems), aquarium accessories (live plants, plastic ornaments, rocks, etc.) in both physical and online pet stores. Consequently, ornamental fish may be kept in any number of ways, some of which seem quite extreme. For example, a popular way of keeping Betta splendens is the so-called Betta-vase. This is usually a decorative vase, in which the animal lives in a very small volume of water without any temperature maintenance, filtration system or vegetation. A similar type of small aquarium is used by pet stores selling male bettas. In this context, the fish are often housed individually in transparent glass jars, smaller than the typical vase. In addition, these jars are placed adjacently. Being in close visual proximity to each other performing aggressive displays to chase the opponent. Shop keepers count on these displays to make the fish more appealing to buyers. The above-mentioned examples touch on potential issues of water quality, available space, exposure to aggressive opponents and the opportunity to hide, and there may be others.

Owing to the growing knowledge of fish physiology and cognitive functions, the general scientific opinion is that fish, like terrestrial animals, are sentient beings; consequently, they are capable of experiencing suffering (Bshary et al., 2002; Laland et al., 2003; Chandroo et al., 2004; Huntingford et al., 2006; Braithwaite and Boulcott 2007; Broom, 2007; Braithwaite et al., 2013). However, disagreement exists on some aspects of this sentiency, like the ability of fish to feel pain (Iwama, 2007; Rose, 2007; Key, 2016). The notion of pain sensation in fish is based on the work by Sneddon (2002; 2003a; 2003b), who identified nociceptors in rainbow trout (Oncorhynchus mykiss) and showed behavioral changes following a painful stimulus in the nociceptors. Although other authors acknowledge the existence of nociception in fish, they argue that fish cannot experience the emotional component of pain, because they lack a neocortex (Iwama, 2007; Rose, 2007; Key, 2016). The recency of the article of Key (2016) and the many reactions in response (Balcombe, 2016; Braithwaite and Droege, 2016; Broom, 2016; Dinets, 2016; Mather, 2016) indicate that this debate is ongoing. Additionally, the study of complex behaviors in fish, like learning through observation, have led to more insight into the cognitive functions of fish. Research has shown that fish are most likely sentient beings, like mammals and birds, because they share the same complex behaviors (Laland et al., 2003; Brandson, 2007; Chandroo et al., 2004). Finally, the growing knowledge of the stress

response and the negative impact of chronic stress in fish have led to the conclusion that fish can suffer due to stress (Mommsen et al., 1999; Chandroo et al., 2004; Huntingford et al., 2006; Iwama, 2007).

Animal welfare has been generally assessed in the literature, in three different ways (Fraser, 2003): (1) the biological functioning of an animal, (2) minimal suffering and the promotion of contentment and (3) the aim to provide a natural life for an animal. Another way to conceptualize animal welfare is by the widely known historic concept of the 'five freedoms', initially put forward by the Brambell committee and later adjusted by the FAWC (farm animal welfare committee), which combines physical health and psychological well-being (FAWC 2009). In this article, the same description of welfare is used as Broom (1986) did, namely that welfare is an individual state as related to its attempts to cope with its environment, in which the three points of Fraser (2003) are included. Despite the diversified scientific literature about fish welfare in general, there are few species-specific welfare data available. The aim of this paper was to examine potential welfare problems in ornamental bettas kept in captivity by combining what is known about the biology of the species and the general information available on housing ornamental fish.

SPECIES DESCRIPTION

Betta splendens, also known as Siamese fighting fish or betta, was first described as a species in 1910 by C.T. Regan (Smith, 1945). Wild fish have a browngreen color and both sexes are about 5 – 5.5 cm in size (Smith, 1945; Jaroensutasinee and Jaroensutasinee, 2001). Domesticated bettas however, are 6 – 6.5 cm (females are a little smaller), and male fish are bred in almost every imaginable color. Females have less bright colors than males (Smith, 1945). There are two varieties of domestic bettas, the betta bred for fighting and the ornamental variety (Smith, 1945; Meejui et al., 2005). To the authors' knowledge, only the ornamental variety with long and colorful fins is kept in Europe.

Bettas are thought to originate from Thailand (Smith, 1945). Their natural habitat exists of shallow ponds and rice paddy fields with plenty of vegetation. This vegetation provides cover against fish-eating birds, like egrets, herons and kingfishers (Jaroensutasinee and Jaroensutasinee, 2001). The water is typically high in temperature, low in oxygen and of low pH (Smith, 1945; Jaroensutasinee and Jaroensutasinee, 2001). Bettas are carnivorous fish with a diet consisting of mosquito larvae and other water insects, which form a source of protein and fat. It is estimated that adult male fish eat about ten to fifteen thousand larvae per year (Smith, 1945; Goldstein, 2004).

Bettas are a member of the family of the Anabantidae, known for their labyrinth organ, which is

a pharyngeal diverticulum that enables consumption of oxygen from the air (Liem, 1963; Kang and Lee, 2010; Alton et al., 2012). Consequently, bettas can survive in water with oxygen levels of as low as 0 - 2 ppm, while other fish die at this concentration (Moore, 1942; Goldstein, 2004). The labyrinth organ is also responsible for the production of air bubbles for nest building. Male bettas build a bubble nest in the centre of their territory, which is both a territorial mark and the site of egg deposition by the female (Braddock and Braddock, 1959; Bronstein, 1982).

The species is well-known and has been much studied for its aggressive and territorial behavior by the male fish, which is also the reason why the species is popular in Asian countries for fighting contests (Smith, 1945; Simpson, 1968; Goldstein, 2004). When placed in the same aquarium, male fish often fight until one dies (Goldstein, 2004).

ENVIRONMENT-RELATED FACTORS

Water quality

One of the most important aspects of fish husbandry is the water quality, characterized by a number of water parameters, such as temperature, concentration of ammonia/ammonium, nitrite and nitrate, and oxygen levels (Hirayama, 1974). Water conditions outside the optimal range have an impact on fish physiology, which could eventually be fatal (Hiramaya, 1974; Hastein et al., 2005; Demeke and Tassew, 2016).

Wild fish are well adapted to the water conditions of their natural environment (Hastein et al., 2005), and the same is true for bettas (Jaroensutasinee and Jaroensutasinee, 2001). Jaroensutasinee and Jaroensutasinee (2001) found that the average water temperature in natural betta habitats during breeding season is $29.9~^{\circ}\text{C} \pm 1.4$, and that pH levels are acidic and range from $5.28{-}5.80$.

For bettas kept as ornamental fish, research about the optimal range of most water parameters is lacking. An indication for the optimal captive water temperature, at least for breeding has been described by Goodrich and Taylor (1934). The authors considered the creation of a bubble nest as a sign of well-being in male bettas and noted that these animals did not build bubble nests when the water temperature was below 24.4 °C or above 27.7 °C. Most bubble nests were built at a temperature of 26.6 °C, leading the authors to conclude this to be the most optimal temperature for breeding in bettas. Although this temperature may first and foremost be optimal for egg development, it is unlikely that the optimal temperature for adult bettas deviates greatly. If temperature would differ significantly, it would be a threat to the survival of particularly the male betta because of prolonged inadequate temperatures. The male would leave the nest exposed to predators and this would eventually result in the extinction of the species.

The nitrogen cycle, in which bacteria in the water convert fish waste products and excess food to ammonia/ammonium, then to nitrite and finally to nitrate (Etscheid, 2003), is another important aspect of water quality. Even low levels of ammonia/ ammonium or nitrite may be lethal to ornamental fish, but no information has been found in the scientific literature about the toxicity levels in bettas. Extrapolation from other fish species is not possible because of the great variability in sensitivity between fish species (Hamlin, 2006; Dolezelova et al., 2011). Also waste products, like uneaten food particles or dead plant leaves, inevitably form in an aquarium. The aerobic breakdown of ammonia and waste products by bacteria lower the amount of dissolved oxygen in the water, which can be potentially lethal (Hirayama, 1974). However, because of the labyrinth organ in bettas, dissolved oxygen might be less of a problem for this species.

In order to manage good water quality, aquarium water should be filtered or changed periodically. A filter physically removes solid waste and provides a surface for bacterial attachment for the biological degradation of waste products and ammonia/ ammonium (Hirayama, 1974). Without added bacteria, the conditioning of a filter takes about 40 - 60 days. After this period, ammonia and nitrite are normally low (Hirayama, 1974). Without a filtration system, water changes are necessary in order to prevent substances to build up (Goldstein, 2004). According to manufacturers of water quality testing products and conditioning products, even nitrate may become toxic in large quantities. However, to the authors' knowledge, no scientific literature exists regarding this topic for home aquaria. Hobbyists literature provides recommendations but these rely on experience. In addition, there is no literature available about the quantity of waste product excretion per betta. This could be interesting to know, in order to calculate the minimum volume of water necessary for a betta given a specific partial water replacement regime. In more recent literature, the impact of chronic doses of nitrite and, more surprisingly, nitrate on health and growth in fish has been described (Voslarova et al., 2008; Bussel et al., 2012; Davidson et al., 2014; Monsees et al., 2016). Moreover, research on production settings of fish has shown that plants can reduce nitrogenous compounds by 25 % (Ng et al., 1990). However, to know the extent of a similar influence in a betta aquarium demands more study.

Food and nutrition

Food and nutrition are essential for the health and survival of ornamental fish (Lewbart, 1998; Mandal et al., 2010). Research has shown that the daily amount of food required to maintain body weight, varies between species, for example < 2.4 % of the body weight in food per day for zebrafish (*Danio rerio*), 1.9 % for neon tetras (*Paracheirodon innesi*), < 1.5 % for moonlight gourami (*Trichopodus microlepis*)

and < 1.0 % for kribensis (*Pelvicachromis pulcher*) (Pannevis and Earle, 1994). Information on the maintenance feeding requirements for the betta, seems to be lacking.

As previously mentioned, wild bettas are carnivorous fish, eating primarily mosquito larvae. However, providing formulated dry food to captive bettas has some benefits because it is less variable in nutritional value and is thus less labor-intensive to maintain good quality. Moreover, it is less costly and there is less risk of pathogen contamination, for example with *Mycobacterium* spp. (Sales and Janssens, 2003; Somsiri et al., 2005; Mandal et al., 2010). However, combining live and formulated food provides better results for growth, reproduction and survival rate of young bettas than a single diet of live or formulated food (Mandal et al., 2010; Puello-Cruz et al., 2010; Sipauba-Tavares et al., 2016).

Both source and quantity of protein in betta food are also important factors. Betta young fed protein of animal origin show better growth and lay more eggs with a higher hatching rate than young raised with food containing proteins of plant origin (James and Sampath, 2003). The quantity of protein in betta food is an even more important factor. Growth in young bettas is optimal when fed food containing 31-35 % protein, but impairs at lower or higher percentages (James and Sampath, 2003; Zuanon et al., 2016). In addition, in female bettas, the ovaries are less developed and eggs are of lesser quality when fed less than 35% protein (James and Sampath, 2003). A protein content of 45% and more is neither favorable for growth or fecundity, possibly due to increased energy expenditure for protein catabolism and increased production of ammonia (James and Sampath, 2003). In 1945, Smith described waste products as growth inhibiting substances and considered them not favorable for propagation of young bettas. When fish are overfed, the excessive excretion of feces and urine combined with uneaten food results in a decreased water quality (Lewbart, 1998). This is also the reason why bettas, and fish in general, need to be fed efficiently, i.e. not too frequently per day and using portions of moderate size (Lewbart, 1998; Sales and Janssens, 2003). For betta young, this means a maximum of two feedings per day (James and Sampath, 2004).

Carotenoids are also important in the food of bettas as they are needed in the immune response. There may also be an interplay with the color of the scales of the fish, as red bettas need carotenoids to maintain their color. When red males are supplemented with carotenoids, their color becomes darker red and their generalized cell mediated immune response is higher. Blue males on the other hand, need few carotenoids for their color and when these males are supplemented with carotenoids, the immune response is even higher than in the red supplemented males (Clotfelter et al., 2007). The increase in immune response by carotenoid supplementation has been found in several

other fish and bird species (Hill, 1999; Blount et al, 2003; Amara et al., 2004; Peters, 2007). However, the question remains if the immune response is lower in red bettas than in blue bettas when fed the same quantity of carotenoids. It is also important to know if the possible lower immunity of red bettas has significant negative health effects.

Aquarium size

Wild bettas have a mean population density of 1.7 animal per square meter (range = 0.5 - 4.8) with a nearly 1:1 M:F sex ratio (Jaroensutasinee and Jaroensutasinee, 2001). The presence of the labyrinth organ in bettas has led to the belief that these fish do not need a large volume of water, since they can extract oxygen from air. However, as oxygen decreases faster in smaller volumes of water, so do waste products accumulate much quicker. Despite other potential factors, like a limited possibility of swimming in small volumes of water, scientific and popular sources use waste product accumulation as the only determinant when recommending aquarium sizes. According to the Ornament Aquatic Trade Association, an aquarium should contain at least four litres of water to prevent the accumulation of toxic products, but this advice is regardless of fish species. When advice is species-specific, it generally remains quite vague. A similarly vague advice for betta, i.e. a large aquarium is preferred over a small one, is given by Goldstein (2004) in his betta handbook.

Social housing and other exposure to conspecifics

Bettas are considered to be territorial fish, particularly the male (Smith, 1945; Simpson, 1968; Goldstein, 2004). In captivity, this may potentially lead to welfare problems due to stress from social interactions when kept with other fish of the same or different species.

Aggressive displays and fighting between male fish are common, even if it is costly from a metabolic point of view (Haller and Wittenberger, 1987; Alton et al., 2012). Male fish produce the endogenic androgen 11-ketotestosterone, responsible for male characteristic development and maintenance (Hackman, 1973). The blood concentration of this hormone rises in aggressive interactions and lowers in non-aggressive interactions (Francis et al., 1993). In other fish species, it has been shown that there is a negative correlation between 11-ketotestosterone and a hormone produced during stressful situations, i.e. cortisol (Pickering et al., 1987; Pottinger et al., 1996). On the other hand, when two betta males interact aggressively while being watched by another male conspecific, the 11-ketotestosteron blood levels rise (Dzieweczynski et al., 2006), implying cortisol levels might be decreased.

A study by Fantino et al. (1972) has indicated that male bettas are highly motivated to perform aggres-

sive displays and that this behavior therefore has a positive valence. Providing access to a mirror image and allowing to perform aggressive displays towards it, could be used as a positive reinforce to teach a male betta to swim through a hoop (Fantino et al., 1972). However, male bettas kept in separate tanks with prolonged visual contact with each other, reduce their aggressive displays over time (Figler, 1972; Peeke and Peeke, 1970). The process takes one to several days, in which every component of the threat display (for example gill cover erection, biting, air gulping) wanes at different times. The question is whether it concerns actual habituation to the sight of the other male betta, or whether other mechanisms, similar to learned helplessness, underlie these findings. Although threat signals may wane following prolonged exposure of male bettas to each other, this does not seem to influence fight readiness or fight outcome (Meliska and Meliska, 1976; Meliska et al., 1980).

Female bettas, with the exception of the 24 hours before mating, prefer not to be kept with a male conspecific and even choose solitude over being in the presence of a male (Snekser et al., 2006). In the presence of a male, the female hides and appears to be intimidated (Bronstein, 1982). When females may choose to be near either a male or a small group of one to three females, they spent more time near the females (Snekser et al., 2006). Although female bettas also fight when placed together in a small aquarium, fighting is more rare, as individual pairs, particularly in larger groups, and they do not have an uninterrupted experience of the aggressive display behavior typically preceding fights (Braddock and Braddock, 1955).

Despite the fact that male bettas are usually housed separately because of their aggressiveness and territorial behavior, it has been documented that a stable community of betta males and females may be established if sufficient space is available (Goldstein, 1975). In a study by Goldsteins (1975), seven males and eight females could be housed in an L- shaped aquarium with arms of 229 x 38 x 51 cm without incidence of severe injuries or complete intimidation.

When multiple fish species are kept together, behavioral changes are seen both at interspecies and at species level (Sloman et al., 2011). It has also been documented that male bettas send aggressive signs to other fish species, which may be followed by an actual fight (Johnson and Peeke, 1972; Johnson and Johnson, 1973; Miley and Burack, 1977). When, during scientific observations, a catfish (Corydoras reticulatus) was briefly attacked by a betta, the attacks quickly ended as it failed to attack back (Johnson and Johnson, 1973). This finding is supported by Braddock and Braddock's (1955) remark that aggressiveness has to be mutual in order to start a fight. In the above mentioned study by Johnson and Johnson (1973), paradise fish (Macropodus opercularis) and dwarf gourami (Trichogaster lalius) had the most vigorous fights with betta, which lasted for an hour.

Environmental enrichment

Environmental enrichment for fish is increasingly gaining interest, particularly for fish typically kept in barren environments, e.g. under laboratory conditions (Williams et al., 2009). The fact that most captive environments do not have the same amount of complexity as the animal's natural habitat may have a negative effect on welfare, as complex environments provide animals with hiding places and, consequently, with a sense of control over their environment (Broom, 1991; Morgana and Tromborg, 2007). In an experimental study in rats by Jay Weiss (1972), it has been demonstrated that environmental control reduces stress in animals even in aversive situations. In fish, this may relate to any stimulus they consider to be a threat, whether it be other fish or the close presence of humans or other pets in the home. Finally, it has been shown that early environmental enrichment may have positive effects on fish later in life, such as the ability to cope better with novel environments, improvement of neuronal plasticity and higher brain mass, including a more developed cerebellum (Kihslinger and Nevitt, 2006; Salvanes et al., 2013; Manuel et al., 2015).

The natural habitat of the betta in Thailand consists of shallow pools with dense vegetation that constitute cover against predators such as fish-eating birds (Smith, 1945; Jaroensutasinee and Jaroensutasinee, 2001). Since even the captive environment consists of stimuli that may be perceived as threatening by fish, for example movement around the aquarium, domestic bettas may also have a need to retreat into vegetation in order to escape these stimuli. It has been documented that zebrafish (Danio rerio) and checker barb (Puntius oligolepis) choose an environment with vegetation when given the choice (Kistler et al., 2011; Schroeder et al., 2014). Because wild zebrafish are equally a prey species and have a similar natural habitat as bettas, it has been suggested that the betta might have the same preference as zebrafish for cover in captivity (Engeszer et al., 2007).

Aside from nutritional needs, food and foraging opportunities may also provide a type of mental stimulation for captive animals (Williams et al., 2009). Contra-freeloading, the phenomenon that an animal favors to work for its food while food is also freely available, has been seen in other animal species (Jensen, 1963; McGowan et al., 2010). When foraging, bettas remain near the water surface to catch prey that reside in the water or have landed on the surface. When they fail to get the prey on the first attack, these food items flee. When such prey is in the water, it attempts to seek cover by diving deeper, while the betta chases it. Given the predatory nature of bettas (Smith, 1945; Paplona et al., 2004), it is likely that this species has a high internal motivation for performing hunting behavior. When they are fed dry food or frozen insects, these items may float on the water surface or sink to the bottom. However, the need for chasing fleeing food disappears. Research on captive carnivorous mammals has shown that providing opportunities to display predatory behavior, reduces the need for coping behavior (as expressed by stereotypies), and is thought to increase welfare (Bashaw et al., 2003).

ANIMAL-RELATED FACTORS

Disease

The authors have opted to focus on diseases, for which, in the literature, the betta in particular have been highlighted. As a result, information on other diseases, such as white spot (*Ichtyophtirius multifiliis*) and fin rot, the common diseases for ornamental fish in general are beyond the scope of this article and can be found elsewhere (Gratzek, 1988; Lewbart, 2001; Roberts et al., 2009; Crim and Riley, 2012).

'Cotton wool' disease, caused by Flavobacterium columnaris, is a bacterial disease, which is especially pathogenic to betta species (Goldstein, 2004). Infected fish show ulcerative lesions with mucus-like filamentous spots (Decostere, 1998; Goldstein, 2004). The bacterium also colonizes the surfaces of the gill, causing necrosis of the extremity of the gill filament, ultimately resulting in progressive necrosis of the entire gill filament (Roberts and Smith, 2011). This causes osmoregulatory problems, which are fatal. The disease can be treated with antibiotics. However, antimicrobial resistance has been documented (Declercq et al., 2013). Prevalence studies in bettas and other fish species are lacking and apart from the publication by Goldstein (2004), there is no literature available about this disease in bettas.

Another condition considered to be important for bettas is 'velvet' disease (Goldstein, 2004). This disease is caused by a dinoflagellate, *Piscinoodinium* spp. Young fry is especially sensitive for infection and the development of clinical disease. The parasite is abundantly present and flourishes in water of poor quality. Skin and gills become infected, resulting in hyperplasia, hemorrhage, osmoregulatory compromise and necrosis (Roberts et al., 2009). Prevention is key for this disease, as fry will mostly die when exhibiting clinical signs (Goldstein, 2004).

Finally, mycobacteriosis is the most common cause of death in bettas on breeding farms in Thailand, and it also poses risks for humans. In fish, it is a slowly progressive disease accompanied by the formation of granulomas in the liver, spleen and kidneys of infected fish (Puttinaowarat, 1999; Zanoni et al., 2008). Other symptoms are extreme anorexia, exophthalmia, keratitis and skeletal deformities (Zanoni et al., 2008; Chansue et al., 2009). In humans, the main clinical signs of this zoonotic disease are limited to skin lesions and ulcers (Gray et al., 1990; Speight and Williams, 1997).

The prevalence of Mycobacterium spp. of domes-

tic bettas in breeding farms in Thailand is 0 - 8 % (Pungkachoboon, 1994). *M. fortuitum* and *M. marinum* are most commonly isolated in bettas (Puttinaowarat, 1999; Puttinaowarat et al., 2002). Zanoni et al. (2008) studied the prevalence of *Mycobacterium* spp. in ornamental fish in Italy, including bettas. In this study, dead or dying fish were selected from home aquaria, with a prevalence of 46.8 %, of which 26 of the 39 examined bettas were positive for some species of *Mycobacterium*. In a study by Prearo et al. (2004), a prevalence of 45 % was found in 312 samples of ornamental fish in Italy. The bettas in that study were commonly infected, but specific data per fish species are absent (Prearo et al., 2004).

Body conformation

The domestic ornamental variety of *Betta splendens*, and more specifically, of the males, is well-known and loved for their long and elegant fins (Smith, 1945; Meejui et al., 2005). Fins are living body parts and contain nerve cells, resulting in pain response when they are damaged (Brandson, 2007). In the scientific literature, no typical fin-associated illness has been found for this species. However, fin damage due to attacks by other fish, e.g. by tiger barbs (*Punctigrus tetrazona*), as is anecdotally known to occur in a multispecies aquarium, is not unlikely.

In addition, the question is whether the long fins themselves hamper the fish to function properly. In other fish species with long tails, such as the montezuma swordtail (Xiphophorus montezumae) and the threatfin rainbowfish (Iriatherina werneri), males with long fins have a higher metabolism (Basolo and Alcaraz, 2003; Trappett et al., 2013). While in guppies (Poecilia reticulata) and threatfin rainbowfish, long fins are not responsible for lower swimming speed, in zebrafish they are (Nicoletto, 1991; Plaut, 2000; Trappett et al., 2013).

DISCUSSION

The aim of this article was to examine if and what potential welfare issues exist for captive bettas. Common practices for keeping bettas, i.e. living in small volumes of water, contact with conspecifics and lack of environmental enrichment were described. Other housing aspects often discussed for ornamental fish keeping in general were highlighted.

Water parameters outside the optimal range have an impact on fish physiology (Hastein et al., 2005; Demeke and Tassew, 2016). The water parameter values found in the natural habitat of bettas seem to match well with the recommendations in the non-scientific literature. The same may not be true however, for the size of the aquarium that is commonly used. Aquarium size recommendations are primarily based on waste product accumulation (ammonia, ni-

trite and nitrate). In the literature, it has been reported that even low doses of these products may have negative impact on fish, particularly in case of ammonia and nitrite, and may be lethal (Voslarova et al., 2008; Bussel et al., 2012; Davidson et al., 2014; Monsees et al., 2016). To the authors' knowledge, no exact toxicity levels for bettas are available, and extrapolation from other species should be exercised with caution. Nitrate susceptibility, for example, seems to vary between fish species (Hamlin, 2006; Dolezelova et al., 2011). This is of absolute importance as many bettas are often kept in small vases without a filtration system. Therefore, water changes are necessary for maintaining good water quality. However, without proper knowledge of the toxicity levels in bettas and the amount of nitrogenous substances produced by the fish, there is no scientific base for recommendations on water change frequency in betta aquaria. Additionally, fluctuations in water parameters, either as a result of water changes or the fast accumulation of waste products in smaller volumes, and their potential effect on welfare should be kept in mind. Finally, live plants might have a stabilizing effect in betta aquaria as they have a reducing effect on nitrogenous compounds (Ng et al., 1990).

Species-adapted nutrition is of the utmost importance for the health and survival of betta fish. In the scientific literature, the combination of live and formulated food has been reported to result in the best growth and survival rate in young bettas (Mandal, et al., 2010; Puello-Cruz et al., 2010; Sipauba-Tavares et al., 2016). If young bettas are fed with formulated food, it should contain 35% proteins, and proteins of animal origin are preferred (James and Sampath, 2003); For betta breeders, there is hence sufficient evidence-based literature on the correct feeding of betta fish. However, for adult bettas and bettas, which are not used for breeding, such feeding guidelines are still lacking. There are betta foods available that meet the nutritional needs of these fish, but they are mostly of a non-living nature. When floating on the surface, such commercial food items allow bettas to attack them from underneath, much like with live prey in natural conditions. Other foods may sink to the bottom, allowing the betta to chase them. However, it remains to be seen whether this mimics natural hunting conditions. To date, there are no data to confirm or disprove an internal motivation for predatory behavior that may be frustrated by captive feeding conditions in captivity.

Certain fish diseases are mentioned as particularly relevant for bettas, such as cotton wool disease and velvet disease (Goldstein, 2004). However, more research is necessary in order to evaluate the exact impact of these two diseases on bettas. Although the cited studies on the prevalence of *Mycobacterium* spp. in Thailand (Pungkachoboon, 1994; Puttinaowarat, 1999; Puttinaowarat et al., 2002; Somsiri et al., 2005) are not quite recent, they provide an indication that *Mycobacterium* spp. might be a serious health issue

for bettas. In a more recent study by Zanoni et al. (2008), quite a number of dead or dying bettas were infected with *Mycobacterium* spp. However, the isolation of *Mycobacterium* in these fish does not automatically mean that this pathogen is the cause of illness or death (Zanoni et al. 2008). More epidemiological research needs to be done on the current prevalence and pathological impact on the betta in order to define the welfare implications.

Social housing is a complex matter in bettas. First, housing males together without continuous conflict may not be feasible unless specific dimensions and aquarium shapes are employed, for example as described by Goldstein (1975). However, it must be noted that this author obtained a stable population of male and female bettas by gradually introducing fish and removing those that, following the first aggressive encounter, became completely intimidated, i.e. remaining immobile in a corner of the aquarium with disappearance of eating behavior). Consequently, during this process, a selection had occurred of a population that could cope with social housing. It also remains to be seen whether the size of the aquarium needed to socially house multiple males and females is realistic for hobbyists. Consequently, unless under specific circumstances, male bettas are best kept physically separate. Secondly, females only seek out betta males a short while before mating, and hide from them all other times (Bronstein, 1982) while preferring to be with other females (Snekser et al., 2006). Hence, it is best not to house males and females together, unless the females are given retreating space and other females live within the group. Thirdly, similar aggression problems between betta males and fish from other species may occur, but data are rather scarce. The size of the aquarium and sufficient vegetation are most likely to be important stabilizing factors (Braddock and Braddock, 1955).

Housing male bettas in separate tanks while allowing them to see each other causes them to frequently perform aggressive displays. Data indicate that being able to see another male betta and threaten it, has rewarding properties. However, the same may not be true for prolonged visual contact. The observation that males with waned threat displays will readily attack other males when placed in a different context (Meliska and Meliska, 1976), could be considered support for the hypothesis stating that a male habituates to the presence of another male. However, an alternative explanation, in line with Weiss' (1972) observations of perceived lack of control by animals over their environment, is that a male -in close and enduring visual contact with another male- experiences increased frustration and eventually stress due to the inability to attain the desired outcome, i.e. chase the opponent. The authors of the present article hypothesize that the observed decrease in threat displays, as reported by several other authors (Figler, 1972; Peeke and Peeke, 1970; Meliska and Meliska, 1976), is the consequence of a rise in cortisol and the associated decrease in 11-ketotestosterone, the latter being the hormone that is related to aggressive behavior. Future research should focus on testing this hypothesis to determine whether enforcing permanent visual contact between male bettas constitutes a welfare problem.

Environmental enrichment, particularly in the form of vegetation that functions as shelter, is also important for betta welfare because of the natural habitat of bettas (Smith, 1945). An enriched environment also allows animals a greater control over their environment, which has been shown in other species to be important to reduce the risk of chronic stress (Weiss, 1972; Morgana and Tromborg, 2007). Finally, there is a positive effect on brain development in young fish (Kihslinger and Nevitt, 2006; Salvanes et al., 2013; Manuel et al., 2015). Although recently, the interest in this topic is increasing for species used in laboratory settings, the effect of barren environments in pet bettas remains to be examined.

Finally, particularly betta males have long fins that may be the target of attacks by other fish. Fins of fish belong to the sensitive body parts (Brandson, 2007), so welfare may be reduced when fins are injured. Data on this topic as well as on the question whether the long fins of ornamental bettas cause problems when functioning (swimming) in water, are clearly lacking. Studies of other species use different methods, have another outcome and can hence not be extrapolated.

CONCLUSION

Aquarium size is a potential welfare issue because of the trend to keep bettas in small vases and the vague water care recommendations primarily based on waste product accumulation. Infection with Mycobacterium spp. is a potential welfare issue because it is abundant in live betta food, betta fish, betta farms and has possible pathologic consequences for infected fish. Female bettas are best kept in groups and without the company of a male, except briefly for breeding purposes. Male bettas are best kept separate from conspecifics (males and females), and housing with other species should be done carefully because fighting can occur. There are indications that prolonged visual exposure to other males could cause welfare issues but more research is required. Vegetation as environmental enrichment in betta aquaria is advisable because their natural habitat consists of thick vegetation to hide from predators. Environmental enrichment may also have a stress-reducing and sheltering effect. However, further research is required in all these domains in order to examine and confirm (or refute) these potential issues.

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REFERENCES

- Alton L.A., Portugal S.J., White C.R. (2012). Balancing the competing requirements of air-breathing and display behaviour during male–male interactions in Siamese fighting fish Betta splendens. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 164*(2), 363–367.
- Amara E.C., Kirona V., Satoha S., Watanabe T. (2004). Enhancement of innate immunity in rainbow Trout (Oncorhynchus mykiss Walbaum) associated with dietary intake of carotenoids from natural products. *Fish & Shell-fish Immunology 16*, 527-537.
- Anderson U.S., Benne M., Bloomsmith M.A., Maple T.L. (2002). Retreat space and human visitor density moderate undesirable behavior in petting zoo animals. *Journal of Applied Animal Welfare Science* 5, 125-137.
- Ang P., Rattana-Apiromyakij N., Goh C.L. (2000). Retrospective study of Mycobacterium marinum skininfections. *International Journal of Dermatology* 39, 343-347.
- Balcombe J. (2016). Cognitive evidence of fish sentience. Commentary on key on fish pain. *Animal Sentience 3*, 8.
- Bashaw M.J., Bloomsmith M.A., Marr M.J., Maple T.L. (2003). To hunt or not to hunt? A feeding enrichment experiment with captive large felids. *Zoo Biology* 22, 189–198.
- Basolo A.L., Alcaraz G. (2003). The turn of the sword: length increases male swimming costs in swordtails. In: *Proceedings of the Royal Society London B* 270, 1631–1636.
- Blount J.D., Metcalfe N.B., Birkhead T.R., Surai P.F. (2003). Carotenoid modulation of immune function and sexual attractiveness in zebra finches. *Science* 300(5616), 125-127.
- Braddock J.C., Braddock Z.I. (1955). Aggressive behavior among females of the Siamese fighting fish, Betta splendens. *Physiological Zoology* 28, 152-172.
- Braddock J.C., Braddock Z.I. (1959). The development of nesting behaviour in the Siamese fighting fish Betta splendens. *Animal Behaviour* 7, 222-232.
- Braithwaite V.A., Boulcott P. (2007). Pain perception, aversion and fear in fish. *Diseases of Aquatic Organisms* 75, 113–131.
- Braithwaite V.A., Huntingford F., Van den Bos R. (2013). Variation in emotion and cognition among fishes. *Journal of Agricultural and Environmental Ethics* 26, 7–23.
- Braithwaite V.A., Droege P. (2016). Why human pain can't tell us whether fish feel pain. Commentary on key on fish pain. *Animal Sentience* 3, 9.
- Brandson E.J. (2007). Fish Welfare. Wiley Blackwell.
- Bronstein P.M. (1982). Breeding, paternal behavior, and their interruption in Betta splendens. *Animal Learning Behavior 10*, 145–151.
- Broom D.M. (1986). Indicators of poor welfare. *British Veterinary Journal* 142, 524–526.
- Broom D.M. (1991). Animal welfare: concepts and measurement. *Journal of Animal Sciences* 69, 4167-4175.
- Broom D.M. (2007). Cognitive ability and sentience: Which aquatic animals should be protected? *Diseases of Aquatic Organisms* 75, 99-108.

- Broom D. (2016). Fish brains and behaviour indicate capacity for feeling pain. Commentary on key on fish pain. *Animal Sentience 3*, 10.
- Bshary R., Wickler W., Fricke H. (2002). Fish cognition: a primate's eye view. *Animal Cognition* 5, 1–13.
- Bussel Van C.G.J., Schroeder J.P., Wuertz S., Schulz C. (2012). The chronic effect of nitrate on production performance and health status of juvenile turbot (Psetta maxima). *Aquaculture 326*, 163–167.
- Chandroo K.P., Duncan I.J.H., Moccia R.D. (2004). Can fish suffer? Perspectives on sentience, pain, fear and stress. *Applied Animal Behaviour Science* 86, 225-250.
- Chansue N., Sermwatanakul A., Anekthanakul K. (2009). *Detection of Mycobacterium in Betta splendens excreta by DNA analysis*. Conference paper, june 2009.
- Chapman F.A., Fitz-Coy S.A. (1997). United States of America trade in ornamental fish. *Journal of the World Aquaculture Society* 28.
- Clotfelter E.D., Ardia D.R., McGraw K.J. (2007). Red fish, blue fish: trade-offs between pigmentation and immunity in Betta splendens. *Behavior Ecology* 18, 1139–1145.
- Crim M.J., Riley L.K. (2012). Viral diseases in zebrafish: what is known and unknown. *Institute for Laboratory Animal Research Journal* 53, 135-143.
- Davidson J., Good C., Welsh C., Summerfelt S.T. (2014). Comparing the effects of high vs. low nitrateon the health, performance, and welfare of juvenile rainbow trout Oncorhynchus mykiss within water recirculating aquaculture systems. *Aquacultural Engineering* 59, 30-40.
- Declercq A.M., Boyen F., den Broeck W., Bossier P., Karsi A., Haesebrouck F., Decostere A. (2013). Antimicrobial susceptibility pattern of Flavobacterium columnare isolates collected worldwide from 17 fish species. *Journal of Fish Diseases* 36, 45-55.
- Decostere A., Haesebrouck F., Devriese L.A. (1998). Characterization of four Flavobacterium columnare (Flexibacter columnaris) strains isolated from tropical fish. *Veterinary Microbiology* 62, 35-45.
- Demeke A., Tassew A. (2016). A review on water quality and its impact on fish health. *International Journal of Fauna and Biological Studies* 3, 21-31.
- Dinets V. (2016). No cortex, no cry. Commentary on key on fish pain. *Animal Sentience* 3, 13.
- Dolezelova P., Mácová S., Pištěková V., Svobodová Z., Bedáňová E., Voslářová E. (2011). Nitrite toxicity assessment in Danio rerio and Poecilia reticulata. *Acta Veterinaria BNRO 80(3)*, 309-312.
- Dzieweczynski T.L., Eklund A.C., Rowland W.J. (2006). Male 11-ketotestosterone levels change as a result of being watched in Siamese fighting fish, Betta splendens. General and Comparative Endocrinology 147, 184-189.
- Engeszer R.E., Patterson L.B., Rao A.A., Parichy D.M. (2007). Zebrafish in the wild: a review of natural history and new notes from the field. *Zebrafish 4*, 21-40.
- Etscheid J. (2003). Nitrogen metabolites in ornamental and pond fishkeeping. Origin, elimination and veterinary relevance. *Tierärztliche Praxis Ausgabe Kleintiere Heimtiere 31*, 244-249.
- Fantino E., Weigele S., Lancy D. (1972). Aggressive display in the Siamese fighting fish (Betta splendens). *Learning and Motivation 3*, 457-468.
- FAWC (2009). Independent report. FAWC Report on Farm Animal Welfare in Great Britain: Past, Present and Future. FAWC advice to government and Animal welfare.
- Figler M.H. (1972). The relation between eliciting stimulus

- strength and habituation of the threat display in male Siamese fighting fish, Betta splendens. *Behaviour* 42, 63-96.
- Francis R.C., Soma K., Fernald R.D. (1993). Social regulation of the brain-pituitary-gonadal axis. In: *Proceedings of the National Academy of Sciences USA 90*, 7794-7798.
- Fraser D. (2003). Assessing animal welfare at the farm and group level: the interplay of science and values. *Animal Welfare* 12, 433-443.
- Goldstein S.R. (1975). Observations on the establishment of a stable community of adult male and female siamese fighting fish (Betta splendens). *Animal Behaviour 23*, 179–185.
- Goldstein R (2004). *The Betta Handbook*. Barrons educational series Inc., New York.
- Goodrich H. B., Taylor H. C. (1934). Breeding reactions in Betta splendens. *Copeia* 4, 165-166.
- Gratzek J.B. (1988). Parasites associated with ornamental fish. *Veterinary Clinics of North America: Small Animal Practice* 18, 375-399.
- Gray S.F., Smith R.S., Reynolds N.J., Williams E.W. (1990). Fish tank granuloma. *British Medical Journal* 300, 1069–1070.
- Hackman E. 1973. Einfluss von Androgenen auf die Geslechtsdifferenzierung verschiedener Cichliden (Teleostei). *General and Comparative Endocrinology*, 21, 44-52.
- Haller J., Wittenberger C. (1987). Biochemical energetics of hierarchy formation in Betta splendens. *Physiology & Behavior 43*, 447-450.
- Hamlin H.J. (2006). Nitrate toxicity in Siberian sturgeon (Acipenser baeri). *Aquaculture 253*, 688–693.
- Hastein T., Scarfe A.D., Lund V.L. (2005). Science-based assessment of welfare: aquatic animals. *Revue Scientifique et Technique-Office International des Epizootis 24*, 529-547.
- Hill G.E. (1999). Is there an immunological cost to carotenoid-based ornamental coloration? *The American Naturalist*, 154(5), 589–595.
- Hirayama K. (1974). Water control by filtration in closed culture systems. *Aquaculture 4*, 369-385.
- Huntingford F.A., Adams C., Braithwaite V.A., Kadri S., Pottinger T.G., Sandoe P., Turnbull J.F. (2006). Current issues in fish welfare. *Journal of Fish Biology*, 68, 332-372.
- Iwama G.K. (2007). The welfare of fish. *Diseases of Aquatic Organisms* 75, 155-158.
- James R., Sampath K. (2003). Effect of animal and plant protein diets on growth and fecundity in ornamental fish, Betta splendens (regan). *Israeli Journal of Aquaculture-Bamidgeh* 55, 39-52.
- James R., Sampath K. (2004). Effect of feeding frequency on growth and fecundity in an ornamental fish, Betta splendens (Regan). *Israli Journal of Aquaculture-Bamidgeh*. 56, 138-147.
- Jaroensutasinee M., Jaroensutasinee K. (2001). Bubble nest habitat characteristics of wild Siamese fighting fish. *Journal of Fish Biology* 58, 1311–1319.
- Jensen J.D. (1963). Preference for bar pressing over "free-loading" as a function of number of rewarded presses. *Journal of Experimental Psychology* 65, 451-454.
- Johnson H.G., Peeke H.V.S. (1972). Patterns of intra- and interspecific aggression in labyrinth fish (Belontiidae). *Behavioral Biology* 7, 335-347.
- Johnson R.N., Johnson L.D. (1973). Intra- and interspecific social and aggressive behaviour on the Siamese fighting

- fish, Betta splendens. Animal behaviour 21, 665-672.
- Kang C.K., Lee T.H. (2010). The Pharyngeal organ in the buccal cavity of the male Siamese fighting fish, betta splendens, supplies mucus for building bubble nests. *Zoology Science* 27, 861-866.
- Kihslinger R.L., Nevitt G.A. (2006). Early rearing environment impacts cerebellar growth in juvenile salmon. *The Journal of Experimental Biology* 209, 504-509.
- Kistler C., Hegglin D., Würbel H., König B. (2011). Preference for structured environment in zebrafish (Danio rerio) and checker barbs (Puntius oligolepis). *Applied Animal Behaviour Science* 135, 318–327.
- Laland K.N, Brown C, Krause J. (2003). Learning in fishes: from three-second memory to culture. *Fish and Fisheries 4*, 199-202.
- Lewbart G. (1998). Clinical nutrition of ornamental fish. Seminars in Avian and Exotic Pet Medicine 7, 154-158.
- Lewbart G.A. (2001). Bacteria and ornamental fish. In: Seminars in Avian and Exotic Pet Medicine. WB Saunders. 48-56.
- Liem K.F. (1963). The comparative osteology and phylogeny of the Anabantoidei (Teleostei, Pisces) 30. *Illinois Biological Monographs* 30, 1-149.
- Mandal S.C., Sahu N.P., Kohli Singh M.P., Das P., Gupta S.K., Munilkumar S. (2010). Replacement of live feed by formulated feed: effect on the growth and spawning performance of Siamese fighting fish (Betta splendens, Regan, 1910). *Aquaculture Research* 41, 1707-1716.
- Manuel R., Gorissen M., Stokkermans M., Zethof J., Ebbesson L.O.E., Vis H. van de, Flik G., Bos R. van den. (2015). The effects of environmental enrichment and age-related differences on inhibitory avoidance in zebrafish (Danio rerio Hamilton). *Zebrafish 12*, 152-165.
- Mather J.A. (2016). An invertebrate perspective on pain. Commentary on key on fish pain. *Animal Sentience 3*, 18.
- McGowan R.T.S., Robbins, Alldredge J.R., Newberry R.C. (2010). Contrafreeloading in grizzly bears: implications for captive foraging enrichment. *Zoo Biology* 29, 484-502.
- Meejui O., Sukmanomon S., Na-Nakorn U. (2005). Allozyme revealed substantial genetic diversity between hatchery stocks of Siamese fighting fish, Betta splendens, in the province of Nakornpathom, Thailand. *Aquaculture* 250, 110–119.
- Meliska J.A., Meliska C.J. (1976). Effects of habituation on threat display and dominance stablishment in the Siamese fighting fish, Betta splendens. *Animal Learning & Behavior 4*, 167-171.
- Meliska C.J., Meliska J.A., Peeke H.V.S. (1980). Threat displays and combat aggression in Betta splendens following visual exposure to conspecifics and one-way mirrors. *Behavioral and Neural Biology* 28, 473–486.
- Miley W.M., Burack G. (1977). Strength of aggressive display in Siamese fighting fish (Betta splendens) toward a conspecific, an alien species (Macropodus opercularis), and a mirror image as affected by prior conspecific visual experience. *Behavioral Biology* 21, 267-272.
- Mommsen P.T., Vijayan M.M., Moon T.W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries* 9, 211–268.
- Monsees H., Klatt L., Kloas W., Wuertz S. (2016). Chronic exposure to nitrate significantly reduces growth and affects the health status of juvenile Nile tilapia (Oreochromis niloticus L.) in recirculating aquaculture systems. *Aquaculture Research*, 1-11.

- Moore W.G. (1942). Field Studies on the oxygen requirements of certain fresh-water fishes. *Ecology* 23, 319-329.
- Morgana K.N., Tromborg C.T. (2007) Sources of stress in captivity. *Applied Animal Behaviour Science* 102, 262-302.
- Ng W.J., Sim T.S., Ong S.L., Kho K., Ho L.M., Tay S.H., Goh C.C. (1990). The effect of Elodea densa on aquaculture water quality. *Aquaculture* 84, 267-276.
- Nicoletto P.F. (1991). The relationship between male ornamentation and swimming performance in the guppy, Poecilia reticulata. *Behavioral Ecology and Sociobiology*, 28, 365-370.
- Ornamental Aquatic Trade Association. Internet reference: http://www.ornamentalfish.org/visited: 29 august 2016.
- Pannevis M.C., Earle K.E. (1994). Maintenance energy requirement of five popular species of ornamental fish. *Journal of Nutrition 124*, 2616S-2618S.
- Peeke H.V.S., Peeke S.C. (1970). Habituation of conspecific aggressive responses in the Siamese fighting fish (Betta splendens). *Behaviour 36*, 232-245.
- Peters A. (2007). Testosterone and carotenoids: an integrated view of trade-offs between immunity and sexual signalling. *BioEssays* 29, 427–430.
- Peter K.L. Ng, Tan H.H. (1997). Freshwater fishes of Southeast Asia: potential for the aquarium fish trade and conservation issues. *Aquarium Sciences and Conservation* 1, 79-90.
- Pickering A.D., Pottinger T.G, Carragher J., Sumpter J.P. (1987). The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown Trout, Salmo trutta L. *General and Comparative Endocrinology* 68, 249-259.
- Plaut I. (2000). Effects of fin size on swimming performance, swimming behaviour and routine activity of zebrafish Danio rerio. *Journal of Experimental Biology* 203, 813-820.
- Pottinger T.G., Carrick T.R., Hughes S.E., Balm P.H.M. (1996). Testosterone, 11-Ketotestosterone, and Estradiol -17b modify baseline and stress-induced interrenal and corticotropic activity in trout. *General and Comparative Endocrinology* 104, 284–295.
- Prearo M., Zanoni R.G., Campo Dall'Orto D., Pavoletti D., Florio D., Penati V., Ghittino C. (2004). Mycobacterioses: emerging pathologies in aquarium fish. *Veterinary Research Communications* 28, 315–317.
- Puello-Cruz A.C., Velasco-Blanco G., Martínez-Rodríguez I.E. (2010). Growth and survival of Siamese fighting fish, Betta splendens, larvae at low salinity and with different diets. *Journal of the World Aquaculture Society 41*.
- Pungkachonboon T. (1994). Studies on Mycobacteriosis in Siamese Fighting Fish Betta Splendens Regan. Doctoral dissertation, Universiti Pertanian Malaysia.
- Puttinaowarat S. (1999). *Detection and Characterisation of Aquatic Mycobacterium spp*. Thesis submitted to the University of Stirling for the Degree of Doctor of Philosophy.
- Puttinaowarat S., Thompson K.D., Kolk A., Adams A. (2002). Identification of Mycobacterium spp. isolated from snakehead, Channa striata (Fowler), and Siamese fighting fish, Betta splendens (Regan), using polymerase chainreaction–reverse cross blot hybridization (PCR–RCBH). *Journal of Fish Diseases* 25, 235-243.
- Roberts H.E., Palmeiro B., Weber E.S. (2009). Bacterial and parasitic diseases of pet fish. *Veterinary Clinics of North America: Exotic Animal Practice* 12, 609-638.

- Roberts H.E., Smith S.A. (2011). Disorders of the respiratory system in pet and ornamental fish. *Veterinary Clinics of North America: Exotic Animal Practice* 14, 179-206.
- Rose J.D. (2007). Anthropomorphism and 'mental welfare' of fishes. *Diseases of Auquatic Organisms* 75, 139-154.
- Sales J., Janssens G.P.J. (2003). Nutrient requirements of ornamental fish. *Aquatic Living Resources* 16, 533-540.
- Salvanes A.G.V., Moberg O., Ebbesson L.O.E., Nilsen T.O., Jensen K.H., Braithwaite V.A. (2013). Environmental enrichment promotes neural plasticity and cognitive ability in fish. In: *Proceedings Biological Sciences 280*.
- Schroeder P., Jones S., Young I.S., Sneddon L.U. (2014). What do zebrafish want? Impact of social grouping, dominance and gender on preference for enrichment. *Laboratory Animals* 48, 328-337.
- Simpson M.J.A. (1968). The display of the Siamese fighting fish, Betta splendens. *Animal Behavior Monographs 1*, 1-74.
- Sipauba-Tavares L.H., Appoloni A.M., Fernandes J.B.K., Millan R.N. (2016). Feed of Siamese fighting fish, Betta splendens, (Regan, 1910) in open pond: live and formulated diets. *Brazilian Journal of Biology* 76, 292-299.
- Sloman K.A. Baldwin L., McMahon S., Snellgrove D. (2011). The effects of mixed-species assemblage on the behaviour and welfare of fish held in home aquaria. *Applied Animal Behaviour Science* 135, 160-168.
- Smith H.M. (1945). Fresh Water Fishes of Siam. Smithsonian Libraries.
- Sneddon L.U. (2002). Anatomical and electrophysiological analysis of the trigeminal nerve in a teleost fish, Oncorhynchus mykiss. *Neuroscience Letters* 319, 167-171.
- Sneddon L.U. (2003a). Trigeminal somatosensory innervation of the head of a teleost fish with particular reference to nociception. *Brain Research* 972, 44-52.
- Sneddon L.U. (2003b). The evidence for pain in fish: the use of morphine as an analgesic. *Applied Animal Behav*iour Science 83, 153-162.

- Snekser J.L., McRobert S.P., Clotfelter E.D. (2006). Social partner preferences of male and female fighting fish (Betta splendens). *Behavioural Processes* 72, 38-41.
- Somsiri T., Puttinaowarat S., Soontornwit S., Lacharoje S. (2005). Contamination of Mycobacterium spp. in live feeds. *Diseases in Asian Aquaculture V*, 227-235.
- Speight L.M.D., Williams H.C. (1997). Fish tank granuloma in a 14-month-old girl. *Pediatric Dermatology 14*, 209-212.
- Tlusty M. (2002). The benefits and risks of aquacultural production for the aquarium trade. *Aquaculture 205*, 203-219
- Trappett A., Condon C.H., White C., Matthews P., Wilson R.S. (2013). Extravagant ornaments of male threatfin rainbowfish (Iriatherina werneri) are not costly for swimming. *Functional Ecology* 27, 1034-1041.
- Voslarova E., Pistekova V., Svobodova Z., Bedanova I. (2008). Nitrite toxicity to danio rerio: effects of subchronic exposure on fish growth. *Acta Veterinaria BRNO* 77, 455-460.
- Weiss J.M. (1972). Psychological factors in stress and disease. *Scientific American* 226, 104-113.
- Whittington R.J., Chong R. (2007). Global trade in ornamental fish from an Australian perspective: The case for revised import risk analysis and management strategies. *Preventive Veterinary Medicine 81*, 92-116.
- Williams T.D., Readman G.D., Owen S.F. (2009). Key issues concerning environmental enrichment for laboratory-held fish species. *Laboratory Animals* 43, 107-120.
- Zanoni R.G., Florio D., Fioravanti M.L., Rossi M., Prearo M. (2008). Occurrence of Mycobacterium spp. in ornamental fish in Italy. *Journal of Fish Diseases* 31, 433-441.
- Zuanon J.A.S., Morais J.A., Souza A.P. (2016). Dietary crude protein levels for juvenile Betta. *Boletim do Institudo de Pesca São Paulo 42*, 590-597.