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Cover image: Fisherman pushes his boat along the channels around Inhaca Island, Mozambique (© José Paula)

First records of sponge-associated Actinomycetes from two coastal sponges from Mauritius

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Abstract

Actinobacteria, commonly known as actinomycetes, are often constituents of sponge-associated bacterial communities and are well known producers of bioactive compounds. In the present study, 7 actinomycete species representing 3 genera were successfully isolated from the sponge *Spheciospongia vagabunda* (Ridley, 1884) and *Neopetrosia exigua* (Kirkpatrick, 1900) from Mauritius. *Neopetrosia exigua* hosted a higher actinomycete diversity resulting in 71% of the total number of actinomycete strains recorded. *Streptomyces* sp. and *Micrococcus* sp. were the most common actinomycete genera, both representing 43% of the total actinomycetes isolated from both host sponges. Actinomycete Isolation Agar was the most effective medium for the selection of actinobacteria, yielding in 57% of the total actinobacterial strains isolated. This study is the first to report on sponge-associated actinomycete communities from Mauritius and paves the way for future sponge-associated actinobacterial research in the Mascarene region.

Keywords: *Neopetrosia exigua*, *Spheciospongia vagabunda,* sponge, Actinobacteria, Mauritius

Introduction

The symbiotic relationship between sponges and bacteria is one of the most ancient symbioses known between microbes and metazoan (Wilkinson, 1984). According to Wilkinson (1978), sponge-associated bacteria in certain sponge species can constitute up to 60% of the sponge biomass. Furthermore, some sponge species are also known to feed unselectively on particles up to 50 µm in size which is about the maximum size that their respective pores allow and therefore they feed on a wide array of microorganisms including several bacterial communities (Ribes *et al*., 1999).

Actinomycetes are non-motile slow growing gram positive bacteria which are phenotypically diverse, omnipresent in most natural environments (Goodfellow and Williams, 1983). Mangrove swamps (Sivakumar *et al*., 2005), deep sea (Weyland, 1984), ocean mud (Jie He *et al.,* 2011), hydrothermal vents (Teske *et al.,* 2002) as well as marine invertebrates (Montalvo *et al.,* 2005; Abdelmohsen *et al*., 2010) are all known to harbour significant diversity of actinomycetes. However, according to Kathiresan *et al*. (2008) and Peraud (2006), the distribution and abundance of actinomycetes in the marine environment have not been extensively investigated. Likewise, actinomycetes are also often the constituents of the sponge-associated bacterial communities (Abdelmohsen *et al.,* 2010; Sun *et al.,* 2015). However, the roles of actinomycetes in marine sponges are still relatively unclear. Peraud (2006)

suggested that these microorganisms may be involved in the processing of metabolic wastes, or could also potentially protect the host sponge against predators, diseases and fouling.

Actinomycetes are known for their ability to produce antibiotics (Kathiresan, 2008). It has been reported that 70% of naturally occurring antibiotics are derived from actinomycetes (Pimentel-Elardo *et al*., 2010). Antibiotics such as melanins originate from marine actinomycetes (Zenova, 1965). Other products are enzymes such as protease (Dixit and Pant, 2000), cellulase (Techapun *et al*., 2003) and chitinase (Robbins *et al*., 1988). In the Western Indian Ocean (WIO), sponge-bacteria derived compounds such as pyrroloiminoquinones, and tsitsikammamine A and B have been successfully isolated by Walmsley *et al.,* (2012). This is further supported by other studies where sponge-derived bioactive compounds have been reported (Davies-Coleman, 2010; Beedessee *et al.,* 2012; Tangman *et al.,* 2015).

To date, multiple reports of sponge-associated actinomycetes have come to light (Montalvo *et al.,* 2005; Abdelmohsen *et al.,* 2014). *Gordonia terrae, Gordinia polyisoprenivorans, Micrococcus luteus* and *Branchybacterium conglomeratum* were all isolated from *Xestospongia* sponge species from USA and Indonesia (Montalvo *et al*., 2005). Moreover, 90 actinomycetes including 14 potentially new strains were isolated from 11 sponge species including *S. vagabunda* from Egypt and Croatia (Abdelmohsen *et al.,* 2010). However, given the huge number of marine sponge species [over 15,000 according to Hooper (2000)] represented in our oceans, current investigations on sponge-associated actinomycetes is still considered limited (Yang, 2013).

Actinomycetes from the WIO region have been previously reported from South Africa (Walmsley *et al.,* 2012), Reunion Islands (Gonzalez *et al.,* 2005), Tanzania (Sosovele *et al.,* 2012) and Mozambique (Canedo *et al.,* 2000). However, sponge-associated actinomycetes have only been reported from South Africa (Walmsley *et al.,* 2012) and Mozambique (Canedo *et al.,* 2000) respectively. No such studies have been reported from the Mascarene Islands. Likewise, sponge-associated actinomycetes have never been reported from Mauritius. The present study therefore aimed at describing for the first time the actinobacterial community associated with two coastal sponge species, namely *Spheciospongia vagabunda* (Ridley, 1984) and *Neopetrosia exigua* (Kirkpatric,1900), from Mauritius.

Materials and methods

Sampling

Sponge samples were collected along the west coast of Mauritius by snorkeling and free diving at depths of 1–3 m. *S. vagabunda* samples were collected at a depth of 1 m from the Albion lagoon (20°'12'29.11"S; 57°'24'32.47"E) whereas *N. exigua* samples were collected at depth of 3 m from the lagoon of Trou aux Biches (20°'14'30.77"S; 57°'47'03.54"E).

Isolation of Actinobacteria

Three isolation media, namely Kuster's Agar, Yeast extract-malt extract Agar, and Actinomycete Isolation Agar, were used for the isolation of sponge-associated actinobacteria (Table 1). All media were supplemented with nalidixic acid (25µg/ml), cyclohexamide (100µg/ml), and nystatin (25µg/ml) to facilitate the isolation of slow-growing bacteria (Abdelmohsen *et al*., 2010; Montalvo *et al*., 2005). Sponge samples were rinsed several times in sterile seawater to remove any transient and loosely attached organisms. One section of the samples was then cut into pieces of approximately 1 cm^3 by using a sterile scalpel and then thoroughly homogenized in a sterile mortar with 10 volumes of sterile seawater. The mixture was filtered and the supernatant was diluted in ten-fold series (10^{-1}, 10^{-2} and 10^{-3}). Aliquots (100 µl) from the fold series were subsequently plated out on triplicate agar plates (Abdelmohsen *et al*., 2010; Montalvo *et al*., 2005). Plates were incubated at 30°C for 6-9 weeks.

Genomic extraction and Identification

Each distinct potential actinomycete colony morphotype observed on the isolation plates was picked and re-streaked until pure cultures were obtained. Isolates were then grown in their respective liquid culture for genomic extraction and identification. Isolates grown in liquid cultures were cryopreserved in medium supplemented with 30% glycerol at -80°C. Genomic DNA from each culture was extracted from its respective broth via a DNA Isolation Kit (UltraClean Microbial, Mo Bio Laboratories, Inc.) following manufacturer's instructions (Abdelmohsen *et al*., 2010; Montalvo *et al*., 2005).

16S rRNA genes (approximately 1500 bp) were amplified by polymerase chain reaction (PCR) using the universal primers 27F (GAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) in a Thermocycler (Applied Biosystems). Primers were selected according to the studies of Montalvo *et al.* (2005) and Abdelmohsen *et al.* (2010).

Table 1. Media composition for the isolation of actinomycetes from *Neopetrosia exigua* and *Spheciospongia vagabunda*.

Medium	Composition	References
Kuster's Agar (KA)	Glycerol 10 g, Casein 0.3 g, Potassium Nitrate 2 g, Sodium Chloride 2 g, Magnesium Sulphate 0.05 g, Calcium Carbonate 0.02 g, Ferrous Sulphate 0.01 g, Agar 15 g; 1 L sterile seawater	Sivakumar, 2001
Yeast extract-malt extract Agar (ISP2)	Dextrose 4 g, Yeast extract 4 g, Malt extract 10 g, Agar 15 g; 1 L sterile seawater	Abdelmohsen *et al*., 2010; Montalvo *et al*., 2005
Actinomycete Isolation Agar (AIA)	Sodium Caseinate 2 g, Asparagine 0.1 g, Sodium Propionate 4 g, Dipotassium Sulphate 0.5 g, Magnesium Sulfate 0.1 g, Ferrous Sulphate 1 mg, Agar 15 g; 1 L sterile seawater	Montalvo *et al*., 2005

Cycling conditions were as follows: initial denaturation at 95°C for 2 min, followed by 30 cycles of 95°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1.5 min. A final extension of 10 min at 72°C was performed. The PCR mixture was composed of 5 µl of 10X DreamTaq Green buffer, 5 µl of dNTP mix, 5 µl of each universal primer, 5 µl of DNA template, 0.5 µl of DreamTaq Green DNA polymerase (including 20 mM $MgCl_2$) and 24.5 µl of MilliQ water in a final volume of 50 µl. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Germany) by following the manufacturer's instructions.

Phylogenetic analysis

Sequences were analyzed through the software FinchTV (Geospiza Inc.) and similarity searches were performed for 16S rRNA sequences of the actinomycete strains by applying their sequences to BLAST search of the NCBI (National Centre for Biotechnological Information, USA). Phylogenetic analysis was performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) Version 6 after multiple alignments of data by ClustalW. A phylogenetic tree was reconstructed by using the neighbour-joining algorithm (bootstrap analyses based on 1000 replicates) following the method of Saitou and Nei (1987).

Results

19 potential actinomycete samples were successfully amplified through polymerase chain reaction. BLAST analysis resulted in 7 colonies from the phylum *Actinobacteria* representing 3 genera namely, *Streptomyces* sp., *Micrococcus* sp., and *Brevibacterium* sp., respectively. Other bacterial strains isolated included 7 *Pseudomonas* sp., 3 *Bacillus* sp., 1 *Oceanobacillus* sp. and 1 *Staphylococcus* sp. respectively.

The number of actinomycetes isolated was different among the two host species. The sponge *N. exigua* hosted the highest actinomycete diversity (71% of the total number of isolates). On the other hand, only 2 actinomycete strains were isolated from the sponge *S. vagabunda*. The most common genera were *Streptomyces* sp. and *Micrococcus* sp. respectively.

Actinomycete Isolation Agar was the highest yielding media for the cultivation of actinomycetes resulting in 51% of the total number of actinobacteria isolated, followed by Kuster's Agar resulting in two strains (29% of total number of isolates), and Yeast-Extract Malt-Extract Agar resulting in a single actinomycete strain. Actinomycete Isolation Agar and Kuster's agar proved to be the more reliable media resulting in diversified actinomycete colonies (2 genera each) as compared to Yeast-Extract Malt-Extract agar (1 genus).

Similarities using BLAST search ranged from 69% - 99% as described in Table 2. The maximum similarity (99%) was noted between strain AG, isolated from *N. exigua* and *Micrococcus* sp. (KM886166.1). A 98% similarity was recorded between the cultured strain AL and *Brevibacterium* sp. (KJ534269.1). On the other hand, the minimum BLAST similarity was observed between strain Y (collected from *N. exigua*) and *Streptomyces fradiae* (EF017718.1) isolated from China.

Table 2. Actinomycetes isolated from *S. vagabunda* and *N. exigua* from Mauritius.

Isolate	Closest Species	GenBank No.	Source Sponge	Isolation Medium	Nearest identified relative (BLAST entry)	Similarity to BLAST entry
W	*Streptomyces* sp.	KU981106	*N. exigua*	AIA	*Streptomyces mutabilis* (EU570424.1)	98%
X	*Streptomyces* sp.	KU981107	*S. vagabunda*	ISP2	*Streptomyces diastaticus* (KF447948.1)	97%
Y	*Streptomyces* sp.	KU981108	*N. exigua*	KA	*Streptomyces fradiae* (EF017718.1)	69%
AG	*Micrococcus luteus*	KU981102	*N. exigua*	AIA	*Micrococcus* sp. (KM886166.1)	99%
AI	*Micrococcus luteus*	KU981103	*S. vagabunda*	AIA	*Micrococcus yunnanensis* (JN999896.1)	96%
AJ	*Micrococcus* sp.	KU981104	*N. exigua*	AIA	*Micrococcus luteus* (FJ380993.1)	97%
AL	*Brevibacterium* sp.	KU981105	*N. exigua*	KA	*Brevibacteirum* sp. (KJ534269.1)	98%

Phylogenetic analysis (Fig.1) demonstrated three groups of actinomycetes. The first group consisted of *Streptomyces* sp. and included the strain X (isolated from *S. vagabunda*), strain W and strain Y (both isolated from *N. exigua*). Isolate X incorporated the Streptomyces clade, whereas isolates W and Y formed separated clades within the group. Likewise, in the *Micrococcus* sp. clade, isolate AI was closely related to the other three *Micrococcus* sp. sequences, whereas isolates AJ and AG formed separate clades. In the third group composed of *Brevibacterium* sp. Isolate, AL (isolated from *N. exigua*) was closely associated with *Brevibacterium oceani* (NR042458.1) and *Brevibacterium iodinum* (KF444388.1).

Discussion

The 7 isolates in the present study originated from the order *Actinomycetales. Streptomyces* and *Micrococcus* were the most common genera, and related isolates were mostly associated with the sponge *N. exigua*. Both genera are well known to be prominent in the marine environment and have been reported in Sivakumar *et al.* 2005. *Streptomyces* and *Micrococcus* are also both known symbionts of marine sponges according to Lijun *et al.* (2012). Both genera have previously been isolated from other petrosid sponge species such as *Petrosia* sp. (Khan *et al.,* 2012) and *Petrosia ficiformis* (Chelossi *et al.,* 2004). Moreover, both genera were also reported from the sponge *S. vagabunda* as described by Abdelmohsen *et al.* (2010).

Initially described from terrestrial environments (Tuleva *et al.,* 2009), the genus *Micrococcus* has previously been isolated from the sponge *Xestospongia* sp. (Montalvo *et al.,* 2005) and *Halichondria panicea* (Schneemann *et al.,* 2010). *Micrococcus* sp. associated with the sponge *S. vagabunda* have also been reported from Egypt (Abdelmohsen *et al.,* 2010; Abdelmohsen *et al.,* 2014) suggesting that some selected *Micrococcus* sp. are possibly common bacterial associates of this sponge species.

Likewise, the representation of *Streptomyces* in the present study is also corroborated in other studies. Actinomycetes from this genus have been previously

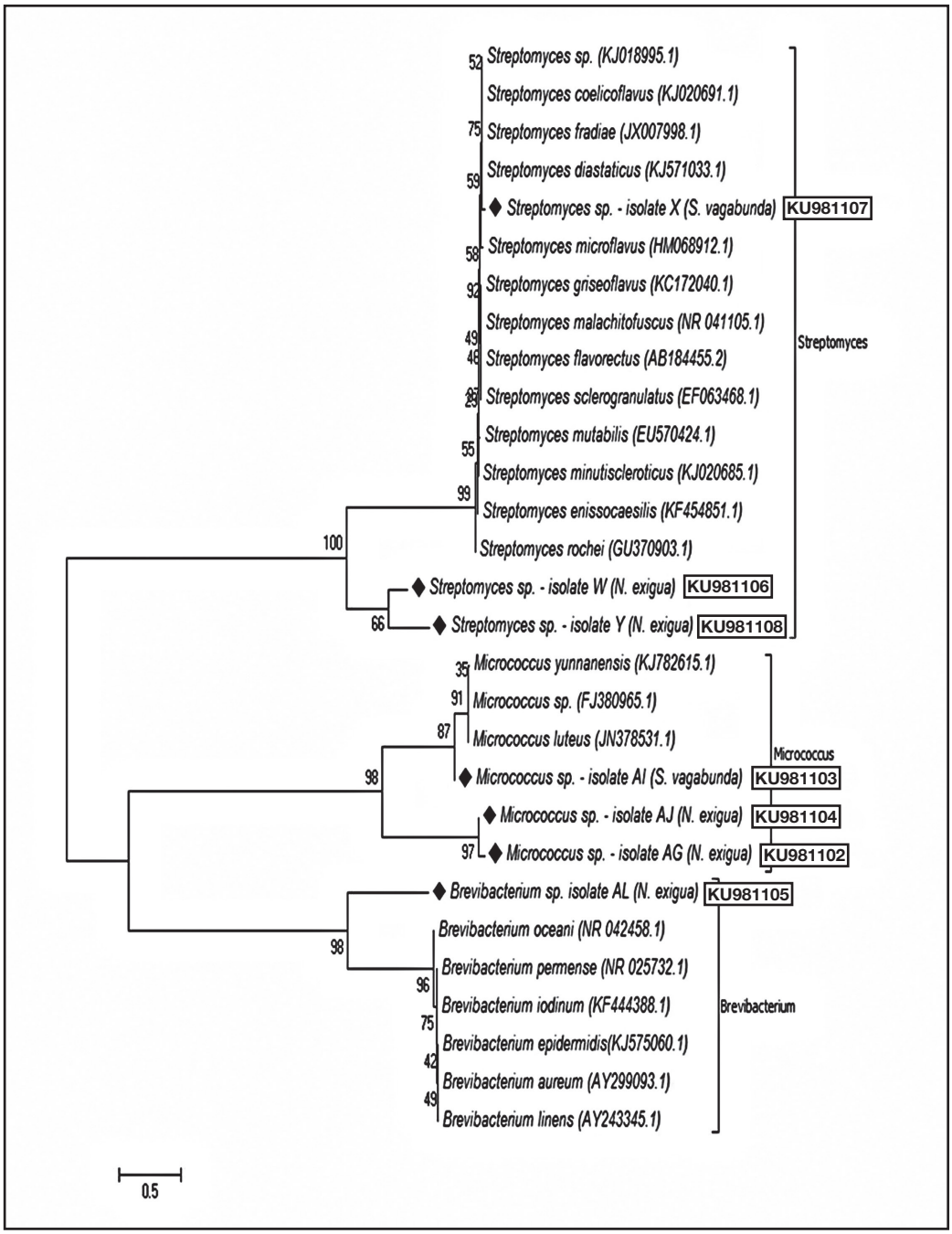

Figure 1. Neighbour-Joining tree of actinomycete strains and their representative species. (♦) denotes the 7 actinobacterial strains identified and their representative species. Scale bar indicates 0.5 substitutions per nucleotide position.

isolated from the sponge *Aplysilla rosea* (Mehbub and Amin, 2012), *Halichondria panicea* (Schneemann *et al.,* 2010) and *Dendrilla nigra* (Selvin *et al.,* 2004). According to Karuppiah (2011) and Abdelmohsen *et al.* (2014), *Streptomyces* are one of the most common genera represented in the marine environment, including sponges (Zhang *et al.,* 2008).

S. vagabunda samples collected from Mauritius resulted in two actinobacterial species, while eleven actinomycetes were previously isolated from *S. vagabunda* in Egypt (Abdelmohsen *et al*., 2010). However, the contrast in the number of isolates could be attributed to the diversity of cultivation media used in the two studies. In the present study, only three cultivation media were used for isolation of actinomycetes, while Abdelmohsen *et al.* (2010) opted for eight different cultivation media. Furthermore, the disparate habitat (or substrate type) of the sponge, and climatic conditions may also significantly influence the diversity and abundance of actinomycetes, as reported by Ghorbani-Nasrabadi *et al.* (2013). The recent study of Abdelmohsen *et al.*

(2014) clearly supports this disparity. While, *Xestospongia testudinaria* collected from Florida (USA) yielded a total of 209 actinomycete strains (Monltalvo *et al.,* 2005), no single strain was found on the same sponge species collected from the Red Sea, Saudi Arabia (Abdelmohsen *et al.,* 2014) confirming that geographical locations might have a major influence on sponge-associated actinomycete diversity.

Actinomycete Isolation Agar was the most prolific cultivation media throughout this study yielding up to four potentially different strains, followed by Kuster's Agar yielding up to two actinobacterial strains of disparate species, while Yeast-Extract Malt-Extract agar (ISP 2) resulted into a single actinomycete (*Streptomyces* sp.) strain. The use of selective media for the isolation of actinomycetes is critical since according to Webster *et al.* (2001), less than 1% of sponge-associated bacteria can potentially be cultivated. Amino acids and protein based media (such as AIA and KA respectively) resulted in a higher number of actinomycetes. In contrast, the lack of additional nutrients in the composition of ISP 2 medium resulted in the isolation of only a single actinomycete strain. The present observation is corroborated by several other studies such as Zhang *et al.* (2008), Selvin *et al.* (2004), Yang (2013) and Oner *et al.* (2014) which highlights the importance of salt, amino acid and protein in actinomycete cultivation media.

The isolation of actinomycetes using AIA and ISP2 is common (Montalvo *et al.,* 2005). In contrast, the successful isolation of actinomycete strains with Kuster's Agar (KA) indicates that glycerol associated with casein as a source of nitrogen enabled the development of actinobacterial micelles and also reduced other bacterial growth (Montalvo *et al.,* 2005). The present results also show some similarities with the studies of Sivakumar (2001) and Sahu *et al.* (2005) where both studies successfully isolated a greater number of actinomycetes using Kuster's Agar.

A relatively low similarity was observed between most of the actinomycete strains isolated in the present study when compared to other sponge-associated actinomycetes on Genbank. Most strains (with the exception of samples AG and AI) correlated with terrestrial actinobacterial strains. This specific observation has previously been reported by Abdelmohsen *et al.* (2010) and Hentschel *et al.* (2002), suggesting that actinomycete communities may not always be sponge-specific as reported.

However, pairwise comparison of *S. vagabunda* associated *Micrococcus* sp. (GU318359) from the study of Abdelmohsen *et al.* (2010) and isolate AI from *S. vagabunda* in the present study showed consequential similarities (96%) with only ten nucleotides gaps. Moreover, a 99% BLAST similarity was observed between isolate AG and *Micrococcus* sp. (KM886166.1) from a non-specified marine sponge in the South China Sea, suggesting that some selected actinomycete species might be sponge-specific. However, additional in-depth research would be necessary to confirm actinomycete species specificity among sponge species.

The low sequence similarities of isolates Y (69%) and AI (96%) suggest that these two strains might belong to novel species. This was graphically supported by the phylogenetic analysis indicating separate clades from these respective genera. However, according to Saitou and Nei (1987), a novel species can only be proposed if a sequence similarity of less than 97% and gaps of less than 2 bp are apparent. With a reported gap of more than 2 bp observed for both isolates, strain Y (81 gaps) and AI (8 gaps) cannot be considered novel species even though the first criteria (< 97% sequence similarity) of Saitou and Nei (1987) was respected.

The present study is a pioneer assessment of sponge-associated actinomycetes in Mauritius. Considering that 70-100% of marine sponges of Mauritius are from off-reef habitats, the discovery of new sponge-associated actinomycete species could potentially play and important role in the development of useful natural products.

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Effect of feeding frequency and feeding rate on growth performance of juvenile silver pompano, *Trachinotus blochii*

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Abstract

The silver pompano *Trachinotus blochii* is ideal species for aquaculture and its success depends on the identification of proper feeding regimens. The objective of this work was to evaluate the ideal feeding rate and frequency for juvenile silver pompano. The experiments were carried out concurrently in a randomized design. A total of 180 fish (7.6 ±0.5g and 10.52±0.01 cm) were stocked in 18 tanks (1000 L) for 8 weeks and fed at 3%, 5%, 10% body weight (BW) per day either in single, or 3x and 6x equal feedings. Weight gain and the specific growth rate increased significantly with feeding rate. The apparent feed conversion ratio showed a significant difference, with the lowest value observed for fish fed 10% (BW/day) in a single feeding. Fish fed at higher feeding rates accumulated significantly more lipid within the body and had associated decreases in moisture, protein, and ash content, but carcass composition was unaffected by feeding frequency. Juvenile pompano show better growth performance when fed 10% BW/day 3 and 6 times a day. It is suggested that the growth of juvenile pompano can be optimized when they are fed at 10% BW/day in three daily feedings.

Keywords: *Trachinotus blochii*, feed management, marine fish culture.

Introduction

Global aquaculture is growing steadily and is feeding an almost equal the number of people as capture fisheries globally with total production reaching an amount of 66.6 million tons in 2012 (FAO, 2014). However, huge continental disparities exist in terms of production, where Asia leads and Africa remains behind despite high demand for food security, producing a mere 1.3 million tons in 2010 (FAO 2012). The demand for fish protein is expected to increase in with the world population growth (FAO, 2009). Good nutrition in animal production systems is essential result in an economically viable healthy product. Nutrition in fish farming is critical because feed presents 40-60% of the production cost (Craig, 2002). Fish nutrition has advanced dramatically in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health. The development of the new species-specific diet formulations support the aquaculture industry as it expands to satisfy increasing demand for affordable, safe and high quality fish and sea food products (Ndome *et al*., 2011).

Development of sustainable aquaculture production depends on various factors such as suitable feeds, culture technology and farming species (FAO, 2014). Despite the technologies available, selection of new fish species with good potential for aquaculture is crucial for the sustainable development of this growing industry (Tutman *et al.,* 2004). The potential species must possess a diverse array of traits to ensure that it is economically viable to farm,

including environmental capability and ecological acceptance (Tutman *et al.,* 2004). Silver pompano (*T. blochii*) has already been considered a suitable candidate for mariculture due to its easy adaptation to culture systems, acceptance of formulated feeds, and fast growth rates (Chavez *et al.,* 2011). The silver pompano is a pelagic and active species that is easy to domesticate and culture in tropical and subtropical marine waters. The pompano species tolerate a wide range of salinities (McMaster *et al.,* 2004), are resistant to low dissolved oxygen and handling stress, readily consume pelleted rations, successfully breed in captivity (Weirich, 2006), and are excellent candidates for aquaculture in a variety of systems (McMaster *et al.,* 2004). However, the specific nutritional requirements of pompano are little known and the available diets mainly consist of fishmeal which accounts for up to 70% of the variable cost (Heilman and Spieler, 1999; Webster *et al.,* 1999).

Several studies have been conducted to assess the culture of this species (Gopakumar *et al*., 2011; 2012; Nazar *et al*., 2012; Kalidas *et al*., 2012). Like other marine species, successful culture of pompano requires high dietary crude protein (CP), with a diet containing 45% CP being the minimum requirement for growth of juvenile pompano (Lazo *et al.,* 1998). Pompano fed with a practical diet with 40% CP resulted in high growth and survival rate, buth poor feed efficiency due to high metabolic rate and poor digestibility (Watanabe, 1995; Lazo *et al.,* 1998). The feed efficiency of juvenile pompano improves when fed with practical diets consisting of 53% CP and 13% crude lipids (CL) at various feeding frequencies (Weirich *et al.,* 2006). Pompano are highly active marine species and it has been suggested that the appropriate diet for successful growth of juvenile pompano requires a high level of digestible energy (DE) to support metabolic and growth demands (Weirich *et al.,* 2006).

Different studies indicate that feeding management practices affect growth and feed conversion ratio of the cultured species (Wang *et al.,* 1998; Cho *et al.,* 2007), and reduce size class variation (Jobling, 1994). Moreover, feeding regimes optimizing feeding frequency and feeding rate may minimize feed wastage and lead to an improvement in environmental safety, greater size-class homogeneity and economic return (Dwyer *et al.,* 2002; Tucker *et al.,* 2006; Cho *at al.,* 2007; Kim *et al.,* 2007; Booth *et al.,* 2008). Insufficient feeding frequency leads to poor growth and high mortality, especially in intensive systems (Carneiro and Mikos, 2005). For example, sporadic feeding and low feeding rates may contribute to reduced growth as well as increased hunger, intraspecific aggression, and increased rate of cannibalism (Folkvord and Ottera, 1993). However, increasing frequency requires more labor and increases production costs (Carneiro and Mikos, 2005). Moreover, fish require food to supply the energy they need for movement and all other functions, and as the "building blocks for growth." The gross energy (or gross calorific value) of food (GE), is the total energy contained in the food and is essential for proper body function. Unfortunately, the maximum growth and the lowest feed conversion ratios do not coincide at the same feeding rate. The lowest feed conversion occurs at feeding rates below those at which maximum growth occurs (De Silva and Anderson, 1995; Goddard, 1996). Thus it is evident that there is a range of possible feeding rates, which depend on whether maximum growth, optimal food conversion, or a balance between the two is sought. Fish carcass composition is a good indicator of physiological condition but it is relatively time consuming to routinely measure (Ali *et al.,* 2006). Feeds and feeding are among the major factors influencing carcass composition and fish quality. Sensory evaluation of fish is an important index in its overall assessment, and determination of the quality of fish. Eating quality therefore is an important determinant of the overall impression of a food (Ochang *et al.,* 2007). Overall, proper feeding frequency and feeding rates vary with fish size, rearing system, temperature and feed quality (Ruohonen *et al.,* 1998; Lovell, 2002). The objective of this work was to evaluate the ideal feeding rate and frequency for juvenile silver pompano.

Methods

Sampling methods

Juvenile silver pompano with an average weight of 7.6g were obtained from Nungwi Beach, which is located at the northern tip of Unguja Island, Zanzibar, and collected using beach seine nets of 2.5 cm mesh size prior to being loaded into 100 L tanks equipped with a supplemental oxygen supply system. Fingerlings were transported early in the morning with the tank tops covered with plastic material to avoid exposure to direct sun light. The tanks were filled with water to 50% of their volume and water exchange was carried out every 30 minutes while fingerlings were transported by boat to the Institute of Marine Sciences Mariculture Center (IMS-MC) at Pangani, Tanga.

Fish were acclimated to the facilities for two weeks and fed with a commercial fish meal diet (crude protein = 50% minimum, crude fat = 11% minimum, crude fiber = 3% maximum, crude ash = 6% maximum; average pellet size = 1mm), to apparent satiation. Subsequently ten fingerlings were stocked randomly into $1m^3$ concrete tanks directly connected to a flow through seawater system, and supplemental aeration provided by a regenerative air blower and air diffusers. Fish were cultured under conditions presumed optimal for silver pompano growth (see water quality information below) and fed available artificial feed at 3, 5 and 10% of body weight BW/day, either in a single feeding (1×) or divided equally among three and six feedings. The feeding frequencies were selected based on about 1% BW per feeding to achieve optimum growth at 1, 3 and 6 feedings per day. While fish are normally fed twice a day, these intervals were changed to elucidate the effect of feeding frequencies on growth rate. Each feeding rate, feeding frequency treatment combination was randomly assigned to three replicate tanks (n = 3). Feeding rates were adjusted to account for growth every 10 days after group- weighing the fish by tank. Fish in the 1× treatments were fed at 13:00, whereas fish in the 3× treatments were fed at 08:00, 13:00 and 18:00, and those in the 6× treatments were fed between 08h00 and 18h00 at 2-hour intervals.

Measurement of environmental parameters

Water quality parameters such as dissolved oxygen (DO), salinity, temperature and pH were measured twice a day for the whole period of the experiment at 09:00 and 16:00 with a WTW multi-parameter probe. Water samples for analysis of ammonium ions were collected twice a week in 250 ml plastic bottles and stored frozen at -20 °C at IMS-MC for the whole experimental period. The samples were then transported in an ice box to the IMS in Zanzibar for analysis. The concentration of ammonia in the water samples was determined as in the UNESCO (1993) protocol. Throughout the experiment, photoperiod was maintained at a 12 h light: 12 h dark cycle, tank inflow rates were maintained at 0.5 L/min, and water quality conditions were maintained as follows (mean ± SD): temperature = 29.6 ± 0.9°C, salinity = 31.1 ± 0.2 g/L, DO = 6.5 ± 0.6 mg/L, total ammonia nitrogen = 0.34 ± 0.08 mg/L, nitrite-nitrogen = 0.35 ± 0.13 mg/L, and pH = 7.61 ± 0.02.

Growth and feed utilization

The total weight gain (TWG), relative growth rate (RGR (%)), specific growth rate (SGR (%/day)), total feed intake (TFI), feed conversion ratio (FCR), protein intake (PI), protein efficiency ratio (PER) and survival (%) were determined according to the methods of De Silva and Anderson (1995). The percentage survival rates were examined based on Jobling (1996).

Proximate analysis

A total of 9 fish per treatment were collected at the end of the experiment, sun dried and frozen at -20°C in preparation for the proximate analysis. The proximate composition of feed ingredients was analyzed at the Department of Animal Science and Production of Sokoine University of Agriculture (SUA) in Morogoro, Tanzania. Crude protein, crude fiber, crude lipid, moisture and ash content were analyzed. Analyses were performed according to standard methods (AOAC, 1995). Moisture content was determined by drying samples in an oven at 105°C to constant weight. Crude lipid was determined using a Soxhlet extractor with petroleum ether (40-60°C boiling range). Crude protein was determined by the Kjeldahl method using digestion block and steam distillation, and ash was determined by incineration of the feed sample in a muffle furnace at 550°C to constant weight.

Statistical analysis

One-way analysis of variance and Duncan's new multiple range tests using the SPSS Statistical Package (SPSS, 21) were carried out to determine if significant differences existed among the means of the above parameters.

Results

The overall mean water quality parameters were typical for these systems. The values of all water quality parameters were consistent and within acceptable ranges for pompano production (Watanabe, 1995). Weight gain, specific growth rate, feed conversion ratio and feed intake increased significantly with feeding rate and feeding frequency. The apparent feed conversion ratio showed significant difference, with the lowest value observed for fish fed 10% BW/day in a single feeding (Table 1).

Growth performance of pompano fingerlings with different feed regimens is presented in Fig. 1. Initially *T. blochii* fingerlings had similar weights and exhibited no significant difference among the treatments ($p < 0.05$). After the 8 week feeding trial, final fish weight and growth generally showed a linear increase with increasing feeding rate (Fig. 1). The highest growth (38.23±0.27) was observed in fish fed six times per

Table 1. Effect of feeding level and feeding rate on growth performance of *T.blochii* during the 8 weeks feeding trial. Results of the One-Way ANOVA test.

Parameters	Feeding level	Feeding frequency	Interaction
Weight gain (g)	$P = 0.013$	$P = 0.04$	$P = 0.003$
Specific growth rate	$P <= 0.004$	$P <= 0.001$	$P = 0.052$
Feed Conversion Ratio	$P = 0.002$	$P = 0.03$	$P = 0.07$
Feed intake	$P = 0.002$	$P = 0.028$	$P = 0.045$

day with 10% BW, followed by (30.337±0.9) in fish fed three times per day at 10% BW, with the lowest value (9.03±0.41) found in fish fed once a day with 3% BW. Fish weight increased significantly over the course of the 50-day experiment, with treatment groups becoming significantly distinct from one another by day 20 (Fig. 1). Weight gain and SGR increased significantly with feeding rate (Fig. 2). Regardless of feeding rate, growth was generally greater and more efficient in the 6× groups than in the 1× groups. The growth-enhancing effect of greater feeding frequency was particularly evident within the 10% BW treatment. Feed intake varied expectedly with feeding rate (Fig. 2). Although feeding rates were constant within individual rate treatments, feed intake expressed as a percentage of body weight was elevated among fish in the 6× group fed at 10% BW relative to the 1× group. Carcass proximate composition was affected by feeding rate, but not by feeding frequency (Table 2). Pompano fed at higher feeding rates accumulated significantly more lipid within the body and had an associated decrease in moisture, protein, and ash content. Significantly highest survival was recorded in fish fed six times a day at 5% BW and those fed a 10% BW/d. There was no significant difference in survival rate among the fish fed at different feed application rates (P<0.05).

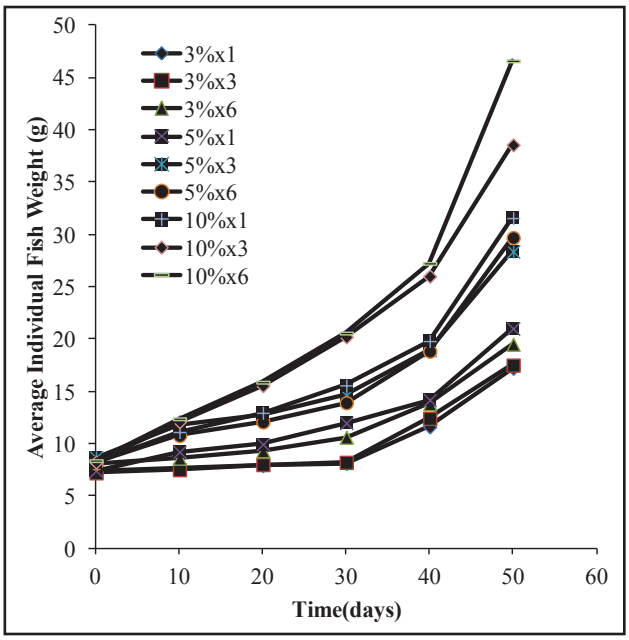

Figure 1. Growth performance and feed utilization of fish feed for different feeding levels and frequency in *T. blochii*.

Discussion

The results from this study indicate that *T. blochii*, fed at the higher rate of 10% a day gain more weight than those fed at a lower rate of 3% a day. Similar observations were reported by Wang *et al.,* (2007) whereby *Nibea miichthioides* fed 1–6% BW/d grew more at the highest feeding rates with less nitrogen retention efficiency, and higher carcass lipid level accumulation. Moreover, related results were observed for cobia (*Rachycentron canadum*) juvenile, which presented a greater SGR when fed with 7% BW/d, rather than with 3% BW/d (Sun *et al.,* 2006). Other comparable findings have been reported for other fish species such as rainbow trout *Oncorhynchus mykiss*, white sturgeon *Acipenser transmontanus*, gilthead seabream *Sparus aurata*, grass carp *Ctenopharyngodon idella*, olive flounder *Paralichthys olivaceus* and Chinese sucker *Myxocyprinus asiaticus* (Storebakken *et al*., 1991; Hung *et al*., 1993; Mihelakakis *et al*., 2002; Du *et al.,* 2006; Kim *et al.,* 2007; Yuan *et al.,* 2010). In the present study, the optimum feeding rate for juvenile silver pompano was 10% BW/d, since growth was lower in fish fed with 3% BW/d, comparable to results reported for *T. marginatus* (Cunha *et al*., 2013) and other tropical fish species including *Clarias gariepinus* (8% BW/d; Marimuthu *et al.,* 2011) and *Colossoma macropomum* (10% BW/d: Silva *et al.,* 2007). These values are higher that subtropical fishes where optimum feeding rates are reported to vary between 2% and 3% BW/d for species such as *Sparus aurata*, *Paralichthys olivaceus* and *Limanda ferruginea* (Mihelakakis *et al.,* 2002; Puvanendran *et al.,* 2003; Kim *et al.,* 2007). The highest values of feeding rate observed in tropical fishes has been suggested to be due to high body metabolic rate (Cunha *et al.,* 2013).

However, while feeding rate has a strong influence on fish growth performance, feeding frequency can

Table 2. Carcass proximate composition of *T.blochii* in the 8-week feeding trial (N=6).

Ingredients %	3	5	10	P value
Moisture	69.3 ± 0.115[a]	66.6 ± 0.208[ab]	63.5 ±0.057[b]	0.0257
Crude Protein	17.3 ± 0.251[a]	16.6 ± 0.251[a]	16.2 ± 0.503[a]	0.0507
Crude Lipid	9.5 ± 0.152[a]	10.6 ± 0.10[ab]	12.3 ± 0.10[b]	0.0273
Ash	3.7 ± 0.404[a]	3.0 ± 0.152[ab]	2.6 ± 0.152[b]	0.0273

[a, b] Treatment means within the same row with different superscript letters are significantly different (P < 0.05)

independently or interactively affect the growth and growth efficiency. The optimum feeding frequency varies from one species to another, and the development of the optimum feeding frequency for specific species depends on several aspects, including culture system, water quality, feed quality and fish development phase (Zuanon *et al.,* 2004). The feeding frequency is higher with no significant reduction of growth rates in fish that are fed at levels below satiation (Ribeiro *et al.,* 2012). It is well known that increasing the feeding frequency tends to increase total feed intake up to a threshold, when fish are fed to apparent satiation (Jobling, 1994). Feeding rate is effectively increased and growth is enhanced with increased feeding frequency, as observed in various species including the Korean rockfish *Sebastes schlegeli*, yellowtail flounder *Limanda ferruginea,* black sea trout *Salmo trutta labrax* and pikeperch *Sander lucioperca* (Lee *et al.,* 2000; Dwyer *et al.,* 2002; Wang *et al.,* 2009). An increase in feeding frequency tends to improve fish growth performance when fish are fed at a fixed rate (Trushenski *et al.,* 2012), and the effect has been found to be diminished to a certain level due to gastrointestinal adaptation in conversion efficiency (Peterson and Small, 2006). The present study has demonstrated that juvenile silver pompano grow better when fed six times a day compared to one or three times. Similar observations were reported by Cunha *et al*. (2013), where the juvenile pompano *T. marginatus* expressed maximum growth when fed eight times daily at a

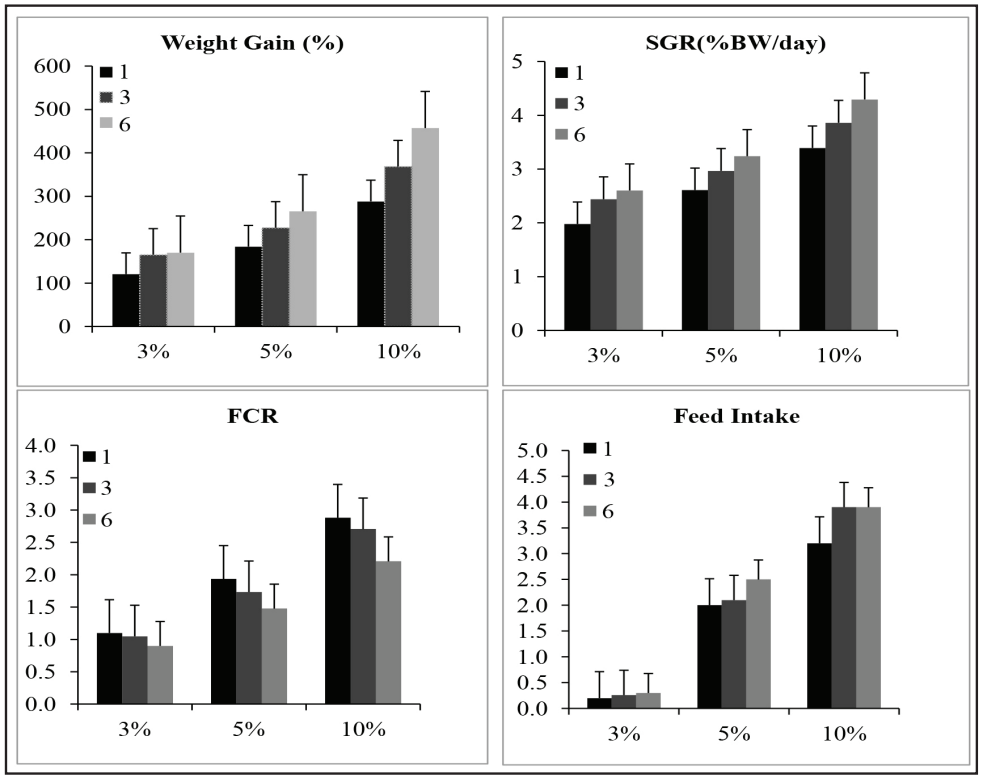

Figure 2. Effect of feeding rate and frequency on weight gain, feed intake, feed conversion ratio and specific growth rate in *T. blochii.*

fixed ratio compared to one or two feedings. Moreover, it has been found that an increase in feeding frequency to ten times daily does not affect growth, but rather increases the value of FCR. Largely consistent results were also reported for red-spotted grouper *Epinephelus akaara* (Kayano *et al.,* 1992), *Plecoglossus altivelis* (Cho *et al.,* 2003), Australian snapper *Pagrus auratus* (Tucker *et al.,* 2006), Asian seabass *Lates calcarifer* (Salama, 2008; Biswas *et al.,* 2010), and Atlantic spadefish *Chaetodipterus faber* (Trushenski *et al.,* 2012). The FCR was highest in the fish fed once daily (1.1, 1.9 and 2.9 respectively for feeding levels of 3%, 5%, and 10% daily).

The result of whole body composition analysis of silver pompano indicates that fish fed at higher feeding rates accumulated significantly more lipid within the body and had associated decreases in moisture, protein, and ash content, but carcass composition was unaffected by feeding frequency. Similar results were reported by Ayo-Olalusi and Ugwumbwa (2009) where *Clarias gariepinus* carcass and muscle components were not affected by feeding frequency. Also Bureau *et al.* (2006) report that fish fed at low feeding levels showed positive protein deposition but negative lipid deposition, suggesting that fish fed at low levels mobilize body lipid reserves to support protein deposition (Table 2).

The growth rate of silver pompano is higher when compared to many other farmed fish. The average total grow-out time from post-hatchery fry to 0.5 kg market-ready fish is about 8 months (Chavez *et al*., 2011). Feeding frequency has a strong influence on fish growth performance and survival rates (Lee *et al.,* 2000; Wang *et al.,* 1998). However, the effect of feeding frequency on survival appears to be species-specific. In the present study, the survival rate was not affected by feeding frequency, similar to what was observed for *Sebastes schlegeli* (Lee *et al.,* 2000) and *T. marginatus* (Cunha *et al.,* 2013). For juvenile *Pagellus erythrinus* survival decreases when feeding frequency is decreased from 4 to 1 times a day (Mihelakakis *et al.,* 2002). Also high survival rates for *T. blochii* grown in cages were reported by Chavez *et al.,* (2011), and lower survival rates were reported by Cremer and Jian (1999) for *T. ovatus* in cages (72%) and by Mc Master *et al*. (2006) in ponds (42%). The high survival rate in the present experiment can be attributed to feeding rate and the species' ability to adapt to manufactured feeds.

Conclusion

Juvenile silver pompano show better growth performance when fed at a feeding rate of 10% BW at a frequency of six times with equal quantities of feed per day. Despite this, the general feeding frequency in commercial production facilities is 2- 3 times per day, probably due to the high of time and labour costs associated with more frequent feeding which will increase production cost.

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