Gene expression of bone morphogenetic proteins and jaw malformation in golden pompano Trachinotus ovatus larvae in different feeding regimes

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**ABSTRACT**

To explore the molecular response of fish larvae to nutritional manipulation, the partial sequences of bone morphogenetic protein (BMP1, BMP2, BMP4, BMP5, and BMP10) genes were obtained, and their expressions were quantified on golden pompano *Trachinotus ovatus* at 28 days post hatching. *Artemia* nauplii were separately enriched with *Nannochloropsis* and Algamac 3080, and non-enriched *Artemia* nauplii were used as a control feed. The lowest jaw deformity rate occurred in fish fed Algamac 3080 enriched *Artemia* nauplii. The expression level of BMP4 of fish fed Algamac 3080 enriched *Artemia* nauplii was significantly lower than those fed *Nannochloropsis* enriched and non-enriched *Artemia* nauplii. The lowest expression level of BMP10 was found in fish fed non-enriched *Artemia* nauplii, and the highest expression of BMP10 was observed in fish fed Algamac 3080 enriched *Artemia* nauplii. Correlation analysis indicates that nutritional manipulations can significantly affect the expression level of BMP4 and BMP10 genes. The concentration of dietary docosahexaenoic acid (DHA) can significantly affect the expression level of BMP10. This study indicates that the use of Algamac 3080 enriched *Artemia* nauplii results in fast fish growth and low jaw deformity, and BMP4 and BMP10 gene expressions were closely related to jaw deformity in golden pompano larvae.

**Introduction**

Skeleton malformation is a major bottleneck continually hindering the production of marine fish fingerlings (Sandel et al. 2010; Cobcroft & Battaglene 2013; Ma et al. 2014c). Malformed fish are usually sold at a low price or are manually removed before sale to market (Ma et al. 2014d). Moreover, deformation in fish can negatively affect fish growth, survival, food conversion ratio, and susceptibility to stress and pathogens (Andrades et al. 1996; Koumoundourous et al. 1997; Boglione et al. 2013). Although genetic factors (Ferguson & Danzmann 1998; Gjerde et al. 2005; Castro et al. 2007; Ma et al. 2014c), environmental factors (Hattori et al. 2004; Sfakianakis et al. 2004; Georgakopoulou et al. 2010; Owen et al. 2012), parasites and pesticides (Liang et al. 2012; Liu et al. 2012) can affect fish bone development, increasingly evidence has showed that nutritional factors during larval fish rearing can directly cause skeleton malformation (Villeneuve et al. 2005a; Mazurais et al. 2009; Darias et al. 2011; Yang et al. 2015).

Skeletogenesis is an important developmental process in vertebrates through which the skeletal development is completed (Grünbaum et al. 2012). During this process, the differentiation and proliferation of different cell types (such as chondrocytes, osteoblasts, osteocytes, and osteoclasts) determine the shape, size and mineral composition of bones (Fernández et al. 2011). The expression of genes associated with skeletal development not only reflects cell proliferation and differentiation, but can be affected by individual genetic characteristics, biotic, and abiotic factors (Cloutier et al. 2010; Fernández et al. 2011; Grünbaum et al. 2012). Therefore, knowledge on gene expression of fish larvae during skeletogenesis is useful to identify the potential cause of skeleton malformation.

Bone morphogenetic proteins (BMPs) belonging to the superfamily of transforming growth factor-β (TGF-β) were originally identified as the molecules that induce ectopic bone formation after implantation into rodent muscles (Wozney et al. 1988; Chen et al. 2004). BMPs are functionally and structurally very conserved throughout animal kingdom, and their biological importance is reflected through functional and structurally redundantness of different BMPs in a single species (Razdorov & Vukicevic 2012). Generally, BMP1 and BMP2 can stimulate osteoblast, and play an important role in bone fracture repair (Grurgievic et al. 2011). BMP2 and BMP4 are involved in skeletogenesis, especially in differentiation of chondrocytes to form cartilage, and both cell differentiation and maturation in the osteoblastic lineage lead to bone formation (Rickard et al. 1994; Minina et al. 2001; Canalis et al. 2003; Wan & Cao 2005).

BMPs play an important role in vertebrate biology, acting as morphogens during embryonic development and as bone inducers, being first recognized for their osteogenic properties (Urist 1965; Wozney et al. 1988). Although several studies have been conducted to analyse the expression of BMP genes in
different fish species, most of these studies focused on fish at the embryonic developmental stage (Myers et al. 2002; Palominco et al. 2014). Knowledge on the expression of BMP genes after hatching and their possible biological significance are very limited in commercially cultured fish species. Up to present, such expression analyses have been only carried out in Atlantic salmon Salmo salar larvae (Ytteborg et al. 2010), and European sea bassDicentrarchus labrax larvae (Villeneuve et al. 2005b, 2006).

Lipid is the main source of energy supply for larval fish (Sargent et al. 1999a, 1999b). The morphogenesis of marine fish larvae can be altered by changing dietary lipids (Cahu et al. 2003). Among different lipid components, fatty acids are indispensable to modulate the transcription of genes involved in metabolism (Kliewer et al. 1997). Although previous studies have demonstrated that feeding with a high level of dietary lipid can improve fish growth performance and reduce skeletal malformation (Cahu et al. 2003; Koven et al. 2003; Izquierdo et al. 2013), excessive dietary lipids and unbalanced fatty acid ratios can also lead to low survival (Fernández & Gisbert 2011; Hamre et al. 2013; Ma & Qin 2014) and skeleton malformation (Izquierdo et al. 2010; Haga et al. 2011; Izquierdo et al. 2013). Nutrient enrichment for Artemia nauplii has been used in larval fish culture for decades, but suitable enrichment formula is not available to lower skeleton malformation in larval fish culture.

Due to high flesh quality, fast growth, and suitability for cage culture, golden pompano has become a good candidate species for aquaculture (Guo et al. 2014). Although several aspects pertaining to hatchery rearing of golden pompano larvae have been well studied (Ma et al. 2014a, 2014b, 2014e), high malformation during early development stage of this species has continually affected its production efficiency in hatchery (Ma et al. 2014d; Zheng et al. 2014). Furthermore, factors causing malformations on this fish are still unclear. Our previous study has found that enriching formula for Artemia nauplii can affect the expression of retinoid X receptors (RXRs) and jaw malformation in golden pompano, but there was no clear correlation between retinoid X receptors (RXRs) expressions and malformation rates when golden pompano larvae were subjected to nutrient change (Yang et al. 2015). In order to further explore the relationships between BMPs gene expression and jaw malformation during the Artemia nauplii feeding phase, we cloned BMPs genes in golden pompano larvae and evaluated the correlation between gene expressions and jaw malformation. Such information would contribute to improvement of larval quality and production efficiency in the aquaculture of golden pompano and other related species.

Materials and methods

The experimental material in present study was collected from an early study on nutritional trial and fish growth measurement, fatty acid analysis, and jaw malformation have been reported in Yang et al. (2015). Fertilized eggs of golden pompano hatched in 500-L fibreglass incubators at 26°C with a hatch rate of 97.1 ± 1.9%. On two days post hatch (DPH), larvae were stocked into four 1000-L larval rearing tanks at a density of 60 fish L−1. Larval rearing tanks were supplied with filtered seawater (5-μm pores) from the bottom of each tank through upwelling with a daily exchange rate of 200% tank volume. Water was discharged through an outlet screen (300 μm) at the top of each tank, and the screen was daily cleaned to reduce clogging. Two air stones were used in each tank to maintain dissolved oxygen close to saturation. Light intensity was maintained at 2400 lux, and the light regime was controlled at 14 h light and 10 h dark. Salinity was maintained at 33 ± 0.8‰ and temperature was at 26.5 ± 1.0°C throughout the experiment. Rotifers Brachionus rotundiformis at a density of 10–20 rotifers mL−1 were used to feed fish larvae from 2 to 12 DPH. On the morning of 11 DPH, fish larvae were restocked into 12 500-L larval rearing tanks at a density of 20 fish L−1.

The nutritional manipulation experiment included three dietary treatments with three replicates each. Artemia nauplii were treated in three methods (1) enriched with instant microalgae paste (Nannochloropsis sp., Qingdao Hong Bang Biological Technology Co., Ltd, Qingdao, China), (2) enriched with Algalmac 3080® (Aquafauna, USA), and (3) without any enrichment as control. For each treatment, three replicate tanks were used in this study, and a total of nine tanks were used in this study. Artemia cysts were produced from the Great Salt Lake, UT, USA (INVE Aquaculture). Artemia nauplii were fed to fish from 11 to 27 DPH. On 11 DPH, Artemia nauplii were first introduced at 200 nauplii L−1, and then added with a daily increment of 90% by number. For the analysis of gene expression, samples were collected from each tank. For each dietary treatment, samples in three replicates were collected.

Fish growth was determined by specific growth rate (SGR) as %/day: SGR = 100 × ([Ln(SL) − Ln(SL)]/Δt, where SL and SL are the final and initial fish total length (mm), respectively, and Δt is the time interval (days) between samples. At the end of this experiment, 50 fish larvae from each tank were sampled for assessing growth and jaw malformation. The remaining fish in each rearing tank were harvested and counted for survival determination.

### Table 1. Summary of genes cloning primers used in this study.

| Primers | Sequence (5′→3′) | Amplicon sizes (bp) |
|---------|------------------|---------------------|
| BMP1-F | TCTCCCCCTCTGTCCTCT | 3238 |
| BMP1-R | CCACCCCAATCCTAACATATA | |
| BMP2-F | CGTGTCGAACGACCTAAC | 1549 |
| BMP2-R | AAAGGGGTGTCCTATAAC | |
| BMP4-F | GACACCTCCCCTTCACAT | 1425 |
| BMP4-R | GAAGCTCCGTTTCAGGTT | |
| BMP5-F | CCAAGGACGACATACAG | 871 |
| BMP5-R | TTAAAGGTTAGCCAGCCTAAT | |
| BMP10-F | GAAGGAGCTCCTCCCCTACA | 1854 |
| BMP10-R | TGCACAATTGCCTTTCTTA | |
| EF-1a-F | TGTTACCTGGCTAGGGG | |
| EF-1a-R | GAGAAAGGACCGCTCA | 1662 |

Note: For BMP names: F, forward primer; R, reverse primer.

Total RNA extraction and reverse transcription

On 28 DPH, approximately 50 individuals were collected from each tank and dissected under a stereo microscope, and jaws were collected for total RNA extraction. Total RNA was extracted using TRIzol (Invitrogen, USA). RNA integrity was verified by electrophoresis on a formaldehyde-agarose gel (1.2%). The RNA concentration was measured by absorbance at 260 nm.
and the purity was determined at the OD 260/280 ratio and agarose gel electrophoresis. RNA was reverse-transcribed to cDNA with oligo (dT) primers using a PrimeScript first strand cDNA synthesis kit (Takara Biotechnology, Dalian Co., Ltd). The cDNA was used as a template in subsequent PCR.

Cloning of the genes cDNA

Based on unpublished golden pompano transcriptome sequences (Illumina HiSeq2000, annotated by NR, KOG, KEGG, and Swissprot), the genes cloning primers were designed (Table 1). The PCR reactions systems were as follows: 1 μL of golden pompano larval cDNA, 1 μL of gene-specific forward primer (F), 1 μL of gene-specific reverse primer(R), 0.5 μL of ExTaq, 5 μL of PCR buffer, 4 μL of dNTP mixture (2.5 μM), 37.5 μL of ddH2O, in a total volume of 50 μL. The PCR conditions were as follows: denaturation at 94°C for 1 min, 35-cycles of 94°C for 30 s, annealing temperature of each genes for 30s, 72°C for 4 min, followed by a 10 min extension at 72°C. The PCR products were cloned into the PMD-19 T vector (Takara, Japan), and sequenced. Identities and positives alignment analysis of BMPs in golden pompano was conducted by using nucleotide BLAST (http://www.ncbi.nlm.nih.gov/).

Gene transcriptional analysis by quantitative real-time PCR

Quantitative real-time PCR (qPCR) was used to analyse the expression levels of BMP genes in golden pompano larva. Gene-specific primer pair for BMP genes (Table 2) were amplified in LightCycler480 II (Roche, Switzerland). The qPCR was performed using SYBR® Premix Ex Taq™ II (Takara, Japan), and EF1α (GenBank Accession No. KT727924) was used as the internal reference and amplified. The 10 μL reaction systems contained 5 μL of 2×SYBR® Premix Ex Taq™ II, 0.4 μL of each forward and reverse primer (10 μM), 1 μL of cDNA template, and 3.2 μL of sterile distilled water. The cycling conditions for BMP genes and EF1α were as follows: 1 min at 95°C, followed by 40-cycles 95°C for 15 s, and 60°C for 1 min. Dissociation curves were employed to ensure that only one single PCR product was amplified in each gene reaction. For each test, three replicates were performed in this study. The relative quantification (RQ) was calculated using the ΔΔCT (comparative threshold cycle) method \( \Delta \Delta CT = CT \text{ of target gene } - \text{ CT of EF-1α} \), \( \Delta \Delta CT \) = ΔCT of any sample - ΔCT of calibrator sample). The efficiencies of the primers (E) were \( E_{BMP2} = 0.998 \), \( E_{BMP4} = 1.004 \), \( E_{BMP5} = 0.923 \), \( E_{BMP10} = 1.004 \).

Fatty acid and jaw malformation analysis

The nutritional content of Artemia nauplii was assessed when fish larvae were at 18 DPH. Fatty acids were analysed at South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, China following the method described by Ma and Qin (2014). Jaw malformation was examined under a stereo microscope (Olympus SZ, Tokyo, Japan) using the criteria described by Ma et al. (2014d). Jaw malformation (%) was calculated as Jaw malformation = (malformed larvae/total larvae) × 100%.

Statistical analysis

The data in this paper were expressed as mean ± SD, and tested by one-way ANOVA (PASW Statistics 18.0, Chicago, SPSS Inc.). When a significant treatment effect was found, Tukey’s test was performed for multiple range comparisons with the level of significant difference set at \( P < .05 \). All the data were tested for normality, homogeneity, and independence to satisfy the assumptions of ANOVA.

Results

Fatty acid compositions of enriched and non-enriched Artemia nauplii are shown in Table 3, which were reported in our previous study (Yang et al. 2015). The highest SGR was obtained in fish fed Artemia nauplii enriched with Algamac 3080 and the lowest SGR was observed in fish fed non-enriched Artemia nauplii. The highest survival was achieved in fish fed non-enriched Artemia or Nannochloropsis enriched Artemia (\( P < .05 \), Table 4), and the lowest survival was observed when fish were fed with Algamac 3080 enriched Artemia nauplii (\( P < .05 \)). On 28 DPH, the jaw deformity of fish larvae fed Algamac 3080 enriched Artemia nauplii was significantly lower than fish fed non-enriched Artemia nauplii or Nannochloropsis enriched Artemia nauplii (\( P < .05 \)). However, the jaw deformity was not significantly different between fish fed non-enriched Artemia nauplii and Nannochloropsis enriched Artemia nauplii (\( P > .05 \)).

Table 2. Summary of qPCR primers used in this study.

| Primers | Sequence(5’–3’) | Amplicon sizes (bp) |
|---------|----------------|---------------------|
| BMP1-qF | CACCATCTCCACACGCTTA | 147 |
| BMP1-qR | AAAGTCGCCGTCGTCGTC | 146 |
| BMP2-qF | CAGGGACAGCTCCGCACAAC | 100 |
| BMP2-qR | TCCCCCGGCTGAAAGG | 101 |
| BMP4-qF | GTGAAACAAACAATCTCCCAAGG | 126 |
| BMP4-qR | GCAGCCCTCCTACCATCATTT | 126 |
| BMP5-qF | GTGGAAGTCTAGACGGCACAA | 100 |
| BMP5-qR | TGGAGAAAGCAACAGGGAAGG | 100 |
| BMP10-qF | CGCGCTACGTCTTCCACACC | 149 |
| BMP10-qR | CGGGTATCCACCACTCCCTCTT | 149 |
| EF1-a-qF | CCCCTGTTCGTTGTTGCC | 101 |
| EF1-a-qR | CGCCTGTTGGTCCTTCCGCTA | 101 |

Note: For BMP names: qF, forward primer for real-time PCR; qR, reverse primer for real-time PCR.
Table 4. SGR, coefficients of variation, final survival rate, and jaw deformity rate of golden pompano larvae fed with enriched and non-enriched Artemia nauplii (Yang et al. 2015). Different letters represent significant differences at P < 0.05.

| Species            | Non-enriched | Nannochloropsis | Algamac 3080 |
|--------------------|--------------|-----------------|--------------|
| SGR (%/day)        | 5.68 ± 0.22a | 6.25 ± 0.08b    | 6.49 ± 0.05a |
| Final survival rate (%) | 31.82 ± 6.60a | 29.33 ± 5.23bH | 30.33 ± 0.90a |
| Jaw deformity rate (%) | 15.91 ± 0.68a | 15.39 ± 0.69b   | 8.33 ± 0.74a |

Table 5. Identities and positives alignment analysis of BMP1 in golden pompano using nucleotide BLAST.

| Species          | Sequence ID | Identities (%) | Positives |
|------------------|-------------|----------------|-----------|
| Larimichthys crocea | KKF14993.1  | 93             | 94        |
| Xiphophorus maculatus | XP_005808545.1 | 96          | 97        |
| Fundulus heteroclitus | XP_012718735.1 | 96        | 97        |
| Danio rerio      | AA65353.1   | 93             | 96        |
| Salmo salar      | NP_001167305.1 | 83         | 90        |
| Oryzias latipes  | XP_004072530.1 | 95         | 97        |
| Maylandia zebra  | XP_012772829.1 | 96         | 97        |

Table 6. Identities and positives alignment analysis of BMP2 in golden pompano using nucleotide BLAST.

| Species            | Sequence ID | Identities (%) | Positives |
|--------------------|-------------|----------------|-----------|
| Paralichthys olivaceus | BAD16743.1  | 95             | 97        |
| Larimichthys crocea | KKF21229.1  | 93             | 95        |
| Sparus aurata      | AA578628.1  | 93             | 95        |
| Oryzias latipes    | NP_001098378 | 84           | 90        |
| Salmo salar        | NP_01167305.1 | 83         | 90        |
| Danio rerio        | NP_571435.1  | 72             | 82        |
| Nototenia coniceps | XP_010772008.1 | 90       | 95        |
| Maylandia zebra    | XP_004542212.1 | 90       | 94        |
| Carassius auratus  | BAN17326.1  | 73             | 82        |
| Astyanax mexicanus | ABK34489.1  | 66             | 75        |

Table 7. Identities and positives alignment analysis of BMP4 in golden pompano using nucleotide BLAST.

| Species            | Sequence ID | Identities (%) | Positives |
|--------------------|-------------|----------------|-----------|
| Cryptococordium simplex | XP_008308407.1 | 97         | 98        |
| Larimichthys crocea | KKF10566.1  | 95             | 96        |
| Boulengerochromis microlepis | BAC02601.1 | 96       | 98        |
| Steatocranus casuarius | BAC02591.1 | 96          | 98        |
| Tropheus duboisi   | BAC02603.1  | 96             | 98        |
| Haplochromis burtoni | BAC02595.1 | 95           | 97        |
| Maylandia zebra    | XP_00454201.1 | 95       | 97        |
| Salmo salar        | NP_001133316.1 | 91       | 94        |
| Salvelinus alpinus  | AFX75411.1  | 89             | 92        |
| Oryzias latipes    | ABK34492.1  | 89             | 94        |
| Danio rerio        | NP_571417.1  | 74             | 83        |

Cloning and expressions of the BMP genes

Partial sequences of BMP1, BMP2, BMP4, BMP5, and BMP10 genes were obtained after sequencing analysis (Appendix 1–5). The BMP1 gene exhibited high identities with other fish species such as Xiphophorus maculatus (96%), Fundulus heteroclitus (96%), and Maylandia zebra (96%, Table 5), while BMP2 showed high identities with Paralichthys olivaceus (95%), Larimichthys crocea (93%), and Sparus aurata (93%, Table 6). BMP4 showed high identities with Cynoglossus semilaevis (97%), Oreochromis niloticus (96%), Boulengerochromis microlepis (96%), and Steatocranus casuarius (96%, Table 7). BMP5 displayed high identities with M. zebra (97%), L. crocea (96%), and F. heteroclitus (96%, Table 8). BMP10 had high identities with O. niloticus (91%), and L. crocea (86%, Table 9).

Nutrient enhancements significantly affected the gene expressions of BMP4 and BMP10 (P < 0.05, Figure 1). The expression of BMP4 in fish fed non-enriched Artemia nauplii or Nannochloropsis enriched Artemia nauplii was significantly higher than fish fed Algamac 3080 enriched Artemia nauplii (P < 0.05, Figure 1). The expression of BMP4 was not significantly different between fish fed non-enriched Artemia nauplii and Nannochloropsis enriched Artemia nauplii (P > 0.05). The lowest level of BMP10 expression was observed when fish were fed with non-enriched Artemia nauplii, and the highest BMP10 expression was found in fish fed Algamac 3080 enriched Artemia nauplii (Figure 1).

The correlation coefficients between nutrients, growth, jaw deformity, and different BMPs expressions are presented in Table 10. Nutrient enhancements were correlated to SGR, jaw deformity, and different BMPs expressions are presented in Table 10. Nutrient enhancements were correlated to SGR, jaw deformity, and different BMPs expressions are presented in Table 10. Nutrient enhancements were correlated to SGR, jaw deformity, and different BMPs expressions are presented in Table 10.

Table 8. Identities and positives alignment analysis of BMP5 in golden pompano using nucleotide BLAST.

| Species            | Sequence ID | Identities (%) | Positives |
|--------------------|-------------|----------------|-----------|
| Cryptococordium simplex | XP_008308407.1 | 97         | 98        |
| Larimichthys crocea | KKF10566.1  | 95             | 96        |
| Boulengerochromis microlepis | BAC02601.1 | 96       | 98        |
| Steatocranus casuarius | BAC02591.1 | 96          | 98        |
| Tropheus duboisi   | BAC02603.1  | 96             | 98        |
| Haplochromis burtoni | BAC02595.1 | 95           | 97        |
| Maylandia zebra    | XP_00454201.1 | 95       | 97        |
| Salmo salar        | NP_001133316.1 | 91       | 94        |
| Salvelinus alpinus  | AFX75411.1  | 89             | 92        |
| Oryzias latipes    | ABK34492.1  | 89             | 94        |
| Danio rerio        | NP_571417.1  | 74             | 83        |

Discussion

Dietary n-3 highly unsaturated fatty acids such as DHA and EPA are essential to growth of fish larvae (Rezek et al. 2010), and their requirements are species-specific (Dantagnan et al. 2010). Fish growth rates are related to dietary DHA in many fish larvae such as gilthead seabream S. aurata (Kovén et al. 1990), red porgy Pago pargo (Roo et al. 2009), yellowtail Seiola quiqueradiata (Furuita et al. 1996), and striped jack Caranx vinctus (Takeuchi et al. 1996). Furthermore, the growth response of fish larvae to different enrichment products is also varied among species. For example, the growth rates of striped bass Morone saxatilis and gilthead seabream S. aurata larvae are not affected by feeding the Artemia nauplii enriched with Algamac 2000 or PL-Cr (DHA-rich phospholipid extract of Cryptothecodinium sp.), but the growth rate of halibut Hippoglossus hippoglossus larvae fed Artemia nauplii enriched with DHA Seleco is lower than the larvae fed PL-Cr (Harel et al. 2002). In this study, fish growth was improved when fish larvae were fed with Artemia nauplii enriched with Algamac 3080 or Nannochloropsis. The best fish SGR was achieved in the treatment of Algamac 3080, which is consistent with the high dietary DHA levels in Artemia nauplii. Nevertheless, the low survival and high coefficients of variation of fish length in the treatment of Algamac 3080 could also contribute to the high SGR due to the death of small larvae in this treatment.

Although a high content of dietary lipid can improve fish survival, overdosed dietary lipid or unbalanced lipid class composition can also lead to poor growth and abnormal development in fish larvae (Salhi et al. 1999; Olsen et al. 2000; Kjørsvik et al. 2009). For instance, Ma and Qin (2014) reported that a high DHA/EPA ratio in live feed could lead to low survival in yellowtail kingfish Seriola lalandi. In the present study, a higher DHA/EPA ratio (0.36:1) was achieved by enriching Artemia nauplii with Algamac 3080. The high DHA/EPA ratio in the Algamac 3080 treatment led to fast fish growth but low survival. In
contrast, better survival was obtained in the non-enriched and \textit{Nannochloropsis} treatments where the DHA/EPA ratio was 0.07:1–0.22:1. Low fish survival in the Algamac 3080 treatment supports the claim that a high DHA content and a high DHA/EPA ratio may reduce larval fish survival (Planas & Cunha 1999) as unbalanced lipid classes in the diet affect digestion and absorption of fatty acids in fish larvae (Salhi et al. 1997; Salhi et al. 1999).

Jaw malformation has been frequently observed in both artificially reared and wild-caught marine fish (Boglione et al. 2013; Ma et al. 2014c). Previous studies have indicated that poly unsaturated fatty acids play an important role in bone formation of fish (Izquierdo et al. 2010, 2013), and dietary fatty acids can alter the composition of bone and cartilage (Kokkinos et al. 1993; Watkins et al. 1997; Liu et al. 2004). Abnormal development of fish larvae may be caused by insufficient dietary n-3 highly unsaturated fatty acids (HUFAs) in live food (Hamre et al. 2002). A 50% reduction of deformed fish was observed when fish larvae were fed with higher levels of dietary DHA (Izquierdo et al. 2010). In our study, fish fed \textit{Artemia} nauplii enriched with Algamac 3080 showed twofold lower jaw malformation than those fed non-enriched \textit{Artemia} nauplii or \textit{Artemia} nauplii enriched with \textit{Nannochloropsis}. Skeletal malformation was reduced in fish fed \textit{Artemia} enriched with Algamac 3080, which is coincident with the high DHA content in the feed. This indicates that a dietary DHA level of 2.56% may be suitable for skeletal development in golden pompano larvae.

BMP1 is an astacin metalloprotease with important cellular functions and diverse substrates (Bond & Beynon 1995; Sterchi et al. 2008). BMP1 plays an essential role in osteogenesis and extracellular matrix, and it can exert influence over the dorsal-ventral structure through an indirect activation of some TGF-β-like proteins (Ge & Greenspan 2006a, 2006b). In zebrfish, BMP1 is a key portion of the chord process activity necessary to the formation of the dorsoventral axis (Jasuja et al. 2006).

BMP2 and BMP4 are closely related proteins involved in key embryonic processes such as dorsal-ventral axis specification (Graff 1997), epithelio-mesenchymal interactions (Vainio et al. 1993), and apoptosis (Graham et al. 1994; Glozak & Rogers 1996; Zou & Niswander 1996). The BMP2 in zebrafish is responsible for induction and maintenance of ventro-lateral cell formation during early development, while a missense mutation in the BMP2b gene can lead to an early dorsalized phenotype in the zebrafish \textit{swirl} mutant, resulting in the lack of cardiogenic mesoderm (Kishimoto et al. 1997). Rafael et al. (2006) suggests that the role of BMP2 during vertebrate development is likely to be part of an ancient mechanism. According to our previous study, the ossification process of golden pompano larvae occurred around 7 DPH, and most structures were completely formed and mineralized by 18 DPH (Zheng et al. 2014). In the present study, the expressions of BMP1 and BMP2 were not significantly affected by the nutrient enrichment by 28 DPH. Results from the present study suggest that the expression of BMP1 and BMP2 in golden pompano may be less sensitive to nutrient enrichment after the bone structure is formed and mineralized.

BMP4 plays diverse roles during vertebrate development, and it is involved not only in the formation of embryonic axis and germ layer induction, but also regulates the formation of tissues and organs (e.g. brain, neural crest, muscle, bone, and cartilage) (Hogan 1996; Mehler et al. 1997; Whitman 1998; Dale & Johns 1999; Shi & Massague 2003). Thus, it has been used to evaluate the effect of micro-nutrients on the skeletal development of marine fish larvae (Villeneuve et al. 2005a, 2005b, 2006). Based on the assumption raised by Villeneuve et al. (2006), the increase in BMP4 and RARγ expression reduces the number of osteoblasts available for bone formation and the loss of bone cells is counterbalanced by the cooperation between retinoic acid and BMP4. In the present study, the expression levels of BMP4 in fish fed non-enriched \textit{Artemia} nauplii and \textit{Nannochloropsis} enriched \textit{Artemia} nauplii were significantly higher than fish fed Algamac 3080 enriched \textit{Artemia} nauplii. Furthermore, jaw malformation in the treatment of non-enriched and \textit{Nannochloropsis} was significantly

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**Table 8.** Identities and positives alignment analysis of BMP5 in golden pompano using nucleotide BLAST.

| Species                  | Sequence ID   | Identities (%) | Positives |
|--------------------------|---------------|----------------|-----------|
| Larimichthys crocea     | KKF21377.1    | 96             | 97        |
| Maylandia zebra          | XP_004556013.1| 97             | 99        |
| Fundulus heteroclitus    | XP_012709077.1| 96             | 97        |
| Poecilia formosa         | XP_007546217.1| 95             | 97        |
| Oryzias latipes          | XP_004077471.1| 93             | 96        |
| Takifugu rubripes        | XP_003964145.1| 92             | 97        |
| Poecilia reticulata      | XP_008428024.1| 95             | 97        |
| Danio rerio              | NP_957345.1   | 86             | 91        |

**Table 9.** Identities and positives alignment analysis of BMP10 in golden pompano using nucleotide BLAST.

| Species                  | Sequence ID   | Identities (%) | Positives |
|--------------------------|---------------|----------------|-----------|
| Larimichthys crocea     | KKF13155.1    | 86             | 93        |
| Takifugu rubripes        | XP_011652343.1| 67             | 75        |
| Oreochromis niloticus    | XP_003453958.1| 91             | 89        |
| Haplochromis burtoni     | XP_005941632.1| 80             | 88        |
| Maylandia zebra          | XP_004538127.1| 79             | 87        |
| Poecilia formosa         | XP_007586655.1| 71             | 83        |
| Oryzias latipes          | XP_004704956.1| 66             | 75        |
| Fundulus heteroclitus    | XP_012704614.1| 65             | 76        |
| Takifugu rubripes        | XP_011603243.1| 67             | 75        |
| Danio rerio              | NP_001124072.1| 49             | 65        |
higher than in the treatment of Algamac 3080. These results are consistent with the finding reported by Villeneuve et al. (2006), in which jaw malformation increases when the expression of BMP4 is up-regulated.

Unlike other BMPs, BMP10 is expressed predominantly in the adult heart and to a less extent in the liver and lung (Neuhäusel et al. 1999). During heart development, BMP10 is expressed in the trabeculae, a common ventricular chamber and atrium of the bulbus cordis (Neuhäusel et al. 1999). In zebrafish, a high level of BMP10 expression was reported in the heart and liver, but a low expression in the brain and kidney (Bland 2001). In the present study, nutrient enhancement altered the expression of BMP10 in golden pompano larvae, and the expression of BMP10 was corresponding to jaw malformation.

In summary, nutrient enhancement can affect jaw malformation in fish larvae during the Artemia nauplii feeding phase. Feeding golden pompano larvae with enriched Artemia nauplii significantly reduced the jaw malformation rate, but also decreased survival at the same time. Reduction of jaw malformation may be due to the mortality of fish larvae during test time. However, this may need further verification. Nutritional manipulations can significantly affect the expression levels of BMP4 and BMP10, and the concentration of dietary DHA can significantly affect the expression of BMP10. The expressions of BMP4 and BMP10 varied between different dietary treatments, and the expressions of BMP4 and BMP10 correspond to jaw malformation in golden pompano larvae during the Artemia nauplii feeding phase. This study suggests that measures of BMP4 and BMP10 in golden pompano may serve as a suitable indicator for jaw malformation in the field and aquaculture facility, leading to rapid assessment of nutrient status affecting fish jaw malformation.

Disclosure statement
No potential conflict of interest was reported by the authors.

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Table 10. Spearman rank correlation coefficients among the response variables.

| Treatment | SGR | Jaw deformity | DHA | EPA | DHA/EPA | BMP1 | BMP2 | BMP4 | BMP5 | BMP10 |
|-----------|-----|---------------|-----|-----|---------|------|------|------|------|-------|
| SGR       | 1.00| −0.80**       | 0.77*| 0.35| 0.65    | 0.08 | −0.28| −0.09| 0.34| 0.80*  |
| Jaw deformity| 1.00| −0.87***      | −0.83**| −0.27| −0.01   | 0.51 | 0.73*| −0.41| −0.86**| |
| DHA       | 1.00| 0.65          | 0.48 | 0.22 | 0.15    | −0.60| 0.64 | 0.80**| 0.59 | |
| EPA       | 1.00| −0.22         | −0.14| −0.54| −0.63   | 0.41 | 0.43 | 0.59 | 0.33 | 0.33  |
| DHA/EPA   | 1.00| 0.57          | 0.10 | 0.20 | 0.34    | 0.40 | 0.40 | 0.40 | 0.40 | 0.40  |
| BMP1      | 1.00| −0.16         | −0.04| −0.21| 0.33    | 0.33 | 0.33 | 0.33 | 0.33 | 0.33  |
| BMP2      | 1.00| 0.08          | −0.3 | −0.51| 0.51    | 0.51 | 0.51 | 0.51 | 0.51 | 0.51  |
| BMP4      | 1.00| −0.63         | −0.41| 0.41 | 0.41    | 0.41 | 0.41 | 0.41 | 0.41 | 0.41  |
| BMP5      | 1.00| 0.28          | 0.28 | 0.28 | 0.28    | 0.28 | 0.28 | 0.28 | 0.28 | 0.28  |
| BMP10     | 1.00| 0.00          | 0.00 | 0.00 | 0.00    | 0.00 | 0.00 | 0.00 | 0.00 | 0.00  |

*P < 0.05.
**P < 0.01.

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Appendix 1. Partial sequences of BMP1
Appendix 2. Partial sequences of BMP2

1   CGTCGACCAAGACCTAACCCGAACCTGCCTGCTTGGTGGAGATTATGTTGGCAGAGGGCGATCG 60
61  CGCAAGGGCTATGGCTCTCTGGCTCCCCAAAGAGAAAGCAAGGCCTGGATTANACCAAGAT 120
121  TAATGGGAAAATTATCCGAGCCACAAAGGGGAACAGGACAGCAGCGGCTCCTCTCTGTG 180
181  CAGGGACTCTGACTGATCAGACGCTGCGGCTGTCCTTGAAGCTGATGACGCGCTGA 240

1    MVAVYVRLMVLLA

241  ggtgtttgctgaggattctacagcctccggagaactacttcccaggggctggtgccgcggagaaaattcagga 300
16   VLEGATGLIPEVGRRKRYSE

301  atccgggaacagcagacccggagacacgctgagactctcactcaagagttggacttcggtct 360
361  SGKQTPEQSESFLNEFELRL

501  tcacagactgctgagccctcttacgctgccagcagcaagacgacagctcgtttgaccgcgca 420
561  LNMFGLRRRPTPSKQAYVVPQ

521  gtcacatgtggcactccctacacagcattgacgagcagcacagaccaacacacatgagAG 480
681  YMVDLYRMHSANGDHSTRKR

541  caagagcactgggagaacacagcagtagagcgcagacaagggccaaacagagattaagctt 540
601  KS MPGKHADRAASKANTIRS

641  tcaacgatagctgtgctgagctcagctttccagggctgctaaagccatcaacacacagtt 600
661  HHESMELASLKKGKTQCF

721  cagttgatagtggcctgcgacctccatgtcatacttatagataaatctgagtttggt 780
761  S0DSGPAAFGHRINIYEIFGV

841  ctccactctctagtgattgggaacacccctctcagagcagcactgtgctggacgcacgta 840
901  PSTDGGEPLARLLDTRLYQD

881  cttcattcacaacggtggtggcagattgtgcagcagcagcccagctcttctctgg 900
951  BSLSRWESFDVSPAIVSQWTSG

991  cnaagcccaacacacctccatgctatggtggaggtagactactccacacagagagagggagttgga 960
1051  KGHHGMFVMVEVLHPPEEGMD

1131  tggagagcactggcagacagctagcgtcctctctcagccaccacagatggcgcgggtc 1020
1231  GEHAQRSSRHRVRSRSLSLQD

1191  cagggactctagcctgctgctgctgccctttctgctgtgctgcagtcgctcagcgggcggtg 1080
1251  QSWSWPAQLRPLLLVTYGHDGR

1211  ggaactcagtagctcacaacagcaagaaactcagagcagctcacaacacagcagagaa 1140
1271  DSVLHTREKRQLAALKQRRK315
1331  gcaacagcacaagacacgcagacacgcagctccgtctgtcgtctgactcagttcttgagtcgg 1200
1391  QCNSKASCRRHLYVDSDKVDSV

1451  gttggaagctggagatgtgaggcctgccggtgaGCAACTGACAGTGTTGATCTGAAAAGAAGTG
1511  VEGCGCGR * 422
1551  GCGAAGAAAGACTGAGAGCGCATCACAGGTTTATATGGAACACCGCGT 1549
Appendix 3. Partial sequences of BMP4

1 GACA CTTCCCTTTTACACCTTACCTCCATCTGAGAGATCCTGTACCTCTCTGCAGAT
2 GGTCACTCTCCAACTGTGCTCTCTGGACGCCCCACACACACACTACAGTCTCTGGAAAAC
3 TGTCACCTTGCTCTCCCCAAAACATGGACTGGTTTCCCCATGTTTTATTCTTGACG
4 ACATCAGTTGCTGTAATGCTGATGATGCTATTATTAATAGGCAGATGGCTG
5 G M P N R M L M V I L I C Q Y L L G 19
6 GAGAGTAAACATGTAGTCTGTGATCACTTGAAAGGAAAAGAGAACCCCTGGCCG
7 E N H A S I L I P E G K K K V P G L Q 39
8 AGTGCTGGGCGGCTGAGGCTAAGCAGTAGGAGTCTGAGGAGTAATGGGCTG
9 G R S A A Q S H E L R D P F E A T L L H 59
10 AAGCTGTCGGCTTCATTAGCAGGCAGGCGGCCTATGAGCTTATCCCAGAAGTTG
11 A C T G T G C C T T A N G C G G G G G C G G G G G A C G T G G C T A C C 420
12 M F G L K R R P R S R S T T V P R Y L 79
13 TGCTGACCTCCTATGCTCACATCGGGAGGAGCTGAGGAGCTGAGGACATGACATTG
14 L D L Y R L Q S G E A E E A A G G H D I A 99
15 CTTTGTGATCGAGGTCGACAGCTGCAAGCCGACGAAACTGTAAGGGATTTACCACTTG
16 F E Y P E R S A S R A N T V R G F H H E 119
17 AAGACACATGGAAGACATTGCTCAGAGTGGAGATGAGAGACATGACCTCAGCTG
18 E H M R V H E L D E G T M P L R F L 139
19 GTTCAGACATCCACAGGAGAGCTGCTCTGCTTCGGCGAATTTAGGTCT
20 F N L S S I P E D E L L S S A E L R L Y 159
21 ACCTGCTAGATCAGGCAGGCAGCTGACTCTCTTCATGGCTAGGAGACTTACC
22 R H Q I D E A I A D S L S G E Q G L H R 179
23 GGTAAACGTGTAGGTGTGACGCGCTCAAGGCACAGCTGAGGAGTTACCACTTG
24 L I V Y E V L K P R P G Q L T Q L L 199
25 TGATACGCGGCTGCGCCACATACGTCGCTCAAGGAGAGCTGAGGACATGACCTG
26 D T R L V R H N T S R W E S F D V S P A 219
27 CAGTCTGCTGGATGAGGCCTCAAGCCGCTCAAGATTTAGGTGTGACGCG
28 V L R W T R E R L P N Y G L A E V L H 239
29 ACCCTAAGAGCGCGCAGCGCTCAGAGGTAATGGGACGCTGAGGACATGACCTG
30 L N Q T P R H Q G R H V R I S R S L H Q 259
31 AGGACCTGGTGAGGACTCAAGGAATGCTCAAGGCCCCTCTGCTGTACCTGGTACG
32 E P G E D W E Q L R P L L V T F G H D G 279
33 GAAGGCTACCGCTGACCCGCCAGCCAAGGGCCACCAAAGGAGGTACAC
34 K G H P L T R R T K R S P K Q R G K R 299
35 GCCAACCGAATCGCGCGCCGAGCTGACGTCGCTGAGGAGTTACTG
36 N R N C R H A L Y V D F S D V G W N D 319
37 ACTGAGTTGGACGCGCTCAGTTACAGGTATAATTGCCCAGGAGAAGCCGCTTAC
38 W I V A P P G Y Q A Y Y C H G E C F P P 339
39 CTGTGACCGTAATTACAAGCAAGACAGCTGAGGACATGACCTG
40 L A D H L N S T H N A I V Q T L V N S S 359
41 TGAACACAGCTTACCGACGCTGAGGACATGACCTG
42 N N N I P K A C C V P T E L S A I S M L 379
43 TCTACCTGAGCAAGCAATCGCTGGATGCTCCTAAAGAATACCAAAAGTACGTAGTGAGG
44 Y L D E H D K V V L K N Y Q E M V V E G 399
45 GTCGCTGGTACGCTTAAACACAAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTAAGACTA403
Appendix 4. Partial sequences of BMP5

1 ggt t a cct ccgtgtg cacagccttat cggcagcgccctcttg tnggc acag cc c gc na 60
1 G Y S R V A Q P Y R A A P L L G H S P A 20
61 c tccacag ccaccacag c accaccat ctt c c c cat g g g t g t g g t g g g g t g g t g g t 120
61 L T T A H D T N L D A D M Y S F V 40
121 a a t t a g t g g g a g a g a t a a g a t t t t t c c a a c c a a g a c a c t a c a a g g a t t c c g t 180
181 tttgcctgactcagctcagatggagaggttagcgcacttgtagattggagtt 240
181 L V E K D H K P H R R H Y K E F R 60
241 a a g a c c gc ac g c g c g c g a a c t c a a a t a a t t c t c a g a g t g t t t t c c a t a t a c a g t 300
281 k d r s h a r y d n i t l k v s i y q v 100
301 a t c a a a g a t a c a a c a a a a g a g a g a c a c t c t t t g c t g a c t c c a a a a a g g c c a g 360
321 i k e y q n k d a e t f l l d s k k v q 120
361 g g g c g a t g g g g c t g g t g t g t g g c a t c a c a c g g c a c c a c t g a c t g g g g t a g t 420
361 A S D G G W L V F D I T A T S N H W V M 410
421 a a c c a c a g g a a c t t g g g c t g c a g c t e t g t g g g a c t g t g a g g g g a g a t a t e 480
481 n p q q n l g l l q l c v e t v d g r s i 160
481 a a c t a a a a c t c t g g a a a c t t g g g a g a t g g g c c c a g t c c a a c a g c c c t c c 540
541 n i k s a g i i g r n g p q s k q p f l 180
541 g g t g c t t c t t c c t a a g c c c a c g g g g t g t a c t c t c t g t a g a g c t g t g g g g t a g g 600
601 a a a a a a g a a c c a a c t c a a t c a a t c a c t a c t a c a c a g a g a g a c t c g g g g g c c a a a a 660
661 v a f f k a s g v l l l r s v r a a g g k 220
721 k k n h n r n k s t n q q e s s r a p k 220
721 a c t g g a g a t a c a c a c c c g t g a c a g a c a g c c t g t a g a a a g a c a c t t t t t g t e 720
721 T G D Y N T S E Q K Q A C C K K H E L Y V 240
781 a g c t c t c g a g a t t t t g g g c c g g g g a g a c c t g g g c c c c t c c t t 780
781 s f r d l g w w d w i i a p e g y a f 260
781 t a c t g g a a g t g a n t g c t c t c c a c t c a c c a c a c a c a g c a a n a n a t g c a 840
781 Y C D G C E S F P L N A H M N A T N H A 280
841 a t t t g g a a c c c c t g g t c a t t a a t t t c t g a a a a t g c c a a a a a g c c t g c t g g 900
841 I V Q T L V H L W F P E N V P K P C C A 300
901 c c a a c c a a c a a c a a a c a t c a t c a t c a c t t t t t g a g c a a c c a a c t t a c c t c e t 960
901 P T K L N A I S V L Y F D D S S N V I L 320
961 a a a n a a a c a a a a t a t g a g c c t g t t g g g g a g c c a t t g T G C T G G C T A A C T T 1020
1021 k k y r n m v v r s c g c h * 334
1021 T 1021