Exploring Serotonin in Catch Contractions and Specific Dynamic Action in Postprandial Metabolism: Insights from Aquatic Models

Caroline Zhang, Jack Fallon

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Article Synopsis

Animal model organisms have long been instrumental in unraveling complex biological processes relevant to human physiology. The first study investigates how exercise and food intake affect metabolism, using zebrafish as our model. The second experiment dives into how neurotransmitters like dopamine and serotonin control muscle movements, particularly catch contractions, in mussels. Examining these processes provides insights into how muscle function and metabolism are regulated, potentially shedding light on human health and conservation efforts.

Graphic by Erin Heyeck

Exploring Serotonin in Catch Contractions and Specific Dynamic Action in Postprandial Metabolism: Insights from Aquatic Models

Caroline Zhang*, Jack Fallon*
*Both authors contributed equally to this work

Duke University
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Preface

By investigating the biological processes using aquatic models such as mussels and zebrafish, new pathways can be explored to better understand fundamental aspects of physiology and behavior. In the following chapters, two studies are presented that exemplify the significance of employing aquatic organisms in scientific inquiry. These studies not only provide valuable insights into the specific mechanisms like metabolism and muscle contractions but also offer broader implications for our understanding of human physiology and ecological conservation.

The first chapter explores the relationship between exercise, food intake, and metabolic rate, employing zebrafish as a model organism. This exploration centers on specific dynamic action, a phenomenon that suggests metabolic rate increases following food consumption. It is hypothesized that digestive processes, such as enzyme secretion and nutrient transportation, incur energetic costs, thereby boosting overall energy expenditure. However, the precise extent of these energetic expenditures and the breadth of species affected remain poorly understood. Through the investigation of the impacts of exercise and feeding on metabolic rate, this research aims to better understand the extent of specific dynamic action and its ramifications for energy utilization. In doing so, it offers valuable insights into animal feeding and husbandry practices while providing insight into the energetic demands of digestion.

In the second chapter, the neurobiology of muscle control is investigated, focusing on neurotransmitters such as dopamine and serotonin, utilizing mussels as the model organism. Mussel catch contractions represent a unique physiological adaptation that allows these organisms to sustain muscle contractions for extended periods with minimal energy expenditure, protecting themselves against predation. This mechanism relies on the modulation of neurotransmitters, which signal initiation and relaxation of the neuromuscular contraction pathway. This investigation aims to unravel the intricate neural circuits and signaling pathways involved in modulating muscle function. The study of mussel catch contractions not only unveils the fascinating adaptations of these organisms but also holds promise for biomimetic engineering applications and enhances our understanding of muscle physiology.

Together, these studies underscore the invaluable role of aquatic model organisms in advancing our understanding of physiology, health, and conservation. By leveraging the unique attributes of these organisms, these experiments not only unravel the mysteries of the natural world but also pave the way for innovative discoveries with broad applications.

INTRODUCTION

Studying fundamental physiological processes through model organisms provides a unique window into understanding mechanisms that underpin broader biological phenomena. Two such processes, specific dynamic action (SDA) and muscle contractions, exemplify this approach, offering insights into critical aspects of metabolism and muscle physiology. Investigations into muscle catch contractions observed in mussels, which maintain shell closure over long durations with minimal energy expenditure, offer valuable insights into regulation of muscle function and the interplay between neurotransmitters like dopamine and serotonin and muscle dynamics. Similarly, specific dynamic action, characterized by an increase in metabolic rate following food consumption, sheds light onto the energy utilization processes that is universal in a wide range of organisms. Through the lens of aquatic model organisms, specifically mussels and zebrafish, the mechanisms underlying SDA and muscle contractions can be better understood, providing valuable insights with broad implications for fields ranging from ecology to human health.

Catch Contractions

Although humans require ATP when contracting muscles, organisms like mussels can use catch contractions to hold their shells closed with little to no energy for extended periods (Funabara et al., 2007). Evolutionarily, this is beneficial because it offers long-term protection without significant energy expenditure. When vertebrate muscle cells experience depolarization, Ca^{2+} ions are released into the cell to initiate a cross-bridge cycle. Ca^{2+} binds to troponin, which unblocks myosin binding sites on actin filaments. Once myosin heads are tightly bound to actin, it uses energy from the release of P_i to drive the power stroke. This causes contractions while Ca^{2+} ions are still present in the cell (Malik et al., 2011). However, invertebrate muscles in catch condition can stay contracted in the absence of Ca^{2+}. Catch contractions are modulated by the conformation of the twitchin protein - when dephosphorylated, twitchin binds to actin and myosin to maintain the contracted state; when phosphorylated, it releases myosin from actin and relaxes the muscle (Figure 1A) (Funabara et al., 2007).

When serotonin (5HT) is applied to the mussel muscle cell, it binds to a G-protein coupled receptor that is linked to adenylate cyclase. Adenylate cyclase converts ATP to cAMP, which activates protein kinase A (PKA). PKA phosphorylates twitchin, and the change in twitchin conformation allows myosin to detach from the actin filaments and relaxes the catch contraction (Figure 1A). Adenylate cyclase, an enzyme involved in the phosphorylation process, is sensitive to 5HT and dopamine (DA) (Deterre et al., 1986). Given that 5HT acts as an activator that leads to the phosphorylation of twitchin, DA was hypothesized to similarly relax catch contractions and a combination of 5HT and DA would increase relaxation response.

METHODS

To test this hypothesis, the experiment was set up as indicated in Patek in terms of calibration curve, dissection and force transducer setup (2016). For each trial, a new mussel was induced to catch condition with 5-8 drops of 10^{-3}M acetylcholine (Patek, 2016). Once the muscle is at peak force, 5-8 drops of instant ocean (n=1), 10^{-3}M 5HT (n=3), 10^{-3}M DA (n=3), or a mixture of 10^{-3}M DA and 10^{-3}M 5HT (n=4) were added. Since the muscle did not relax after instant ocean was added in the control trial, the experiment with 5HT was terminated. Relaxation response was measured by how much the muscle relaxes from catch contraction due to the addition of chemicals (labeled as reduction from peak force) as well as the time it takes for this relaxation to occur (labeled as relaxation rate).

RESULTS

Catch contractions were induced in mussels using acetylcholine. After ocean water was added to the mussel in catch condition (as a control), there was no relaxation response. The control mussel showed no reduction from peak force, and thus a relaxation rate of 0N/s. The mussels treated with DA had a mean reduction from peak force that was less than those treated with 5HT, but were greater than control (Figure 1B). Similarly, the mean relaxation rate of DA-treated mussels was slower than that of 5HT-treated mussels (Figure 1C). There was no overlap between relaxation response data of 5HT-treated mussels and DA-treated

mussels – the minimum reduction from peak force of 5HT-treated mussels was greater than the maximum of DA-treated mussels (Figure 1B). The same can be said about the relaxation rate of mussels that were treated with DA versus 5HT (Figure 1C). This shows that DA had a similar, but lesser, effect on relaxation response than 5HT, supporting the hypothesis that DA would also initiate the phosphorylation cascade.

When a 5HT and DA mixture was applied, the reduction from peak force was in between that of 5HT and DA (Figure 1B). Correspondingly, the relaxation rate of mussels treated with 5HT+DA was also in between that of mussels treated with 5HT and DA (Figure 1C). The maximum value for reduction from peak force of 5HT+DA-treated mussels was within the range of 5HT-treated mussel data, while the minimum value of 5HT+DA-treated mussels was within the range of DA-treated mussel data (Figure 1B). Again, data on relaxation rate of 5HT+DA-treated mussels shows the same trend in overlap with 5HT-treated mussels and DA-treated mussels. This does not support the second part of the hypothesis, but could be explained by competition of 5HT and DA in the adenylate cyclase reaction.

DISCUSSION

DA was anticipated to relax catch contractions, as 5HT does. A combination of 5HT and DA was also expected to cause greater amplitude of relaxation from peak contraction and faster relaxation rate than 5HT alone. To assess this hypothesis, instant ocean, 5HT, DA, or a mixture of DA and 5HT were added to mussels in catch condition. The mussels treated with DA had a lower mean reduction from peak force and slower mean relaxation rate than those treated with 5HT, which supports the first part of the hypothesis. When a 5HT and DA mixture was applied, the reduction from peak force and relaxation rate were greater than those of mussels treated with DA, but less than those of mussels treated with 5HT, which differs from the second part of the hypothesis.

5HT is known to relax catch contractions by initiating a metabotropic cascade that results in twitchin phosphorylation – activate adenylate cyclase to generate cAMP, which triggers PKA to phosphorylate twitchin (Figure 1A) (Funabara et al., 2007). When twitchin is phosphorylated, the muscle becomes relaxed. Adenylate cyclase is coupled to both 5HT and DA receptors, which suggests that DA would have a similar effect as 5HT in catch relaxation (Gies, 1986). However, previous literature determined that DA has a lesser effect on muscle relaxation than 5HT, possibly because DA is a weaker activator of adenylate cyclase (Gies, 1986). At various concentrations of 5HT and DA, catch relaxation caused by 5HT occurs faster than catch relaxation caused by DA (Gies, 1986). Additionally, when DA concentration is incremented by an order of magnitude from 10^{-8}M to 10^{-4}M, relaxation response also increases as concentration increases; this indicates some dose-dependency in catch relaxation due to DA (Gies, 1986).

The method for this experiment was based on previous protocols, which found that the most effective and consistent dose of 5HT was 10^{-3}M, so the same dose of DA was used. As mentioned previously, research indicates that the effects of DA are dose-dependent (Gies, 1986). Addition of DA shows less reduction from peak force and slower relaxation rate than 5HT, and it is possible that a greater concentration of DA would produce a stronger response that is more like that of 5HT (Figure 1B). Similarly, greater concentration of DA in the combination of DA and 5HT would generate data with smaller value of standard deviation, since higher concentration of DA might increase relaxation response to be comparable that of 5HT. Testing a range of dosages and concentrations of DA would offer better insight into the mechanism of how DA relaxes catch contractions.

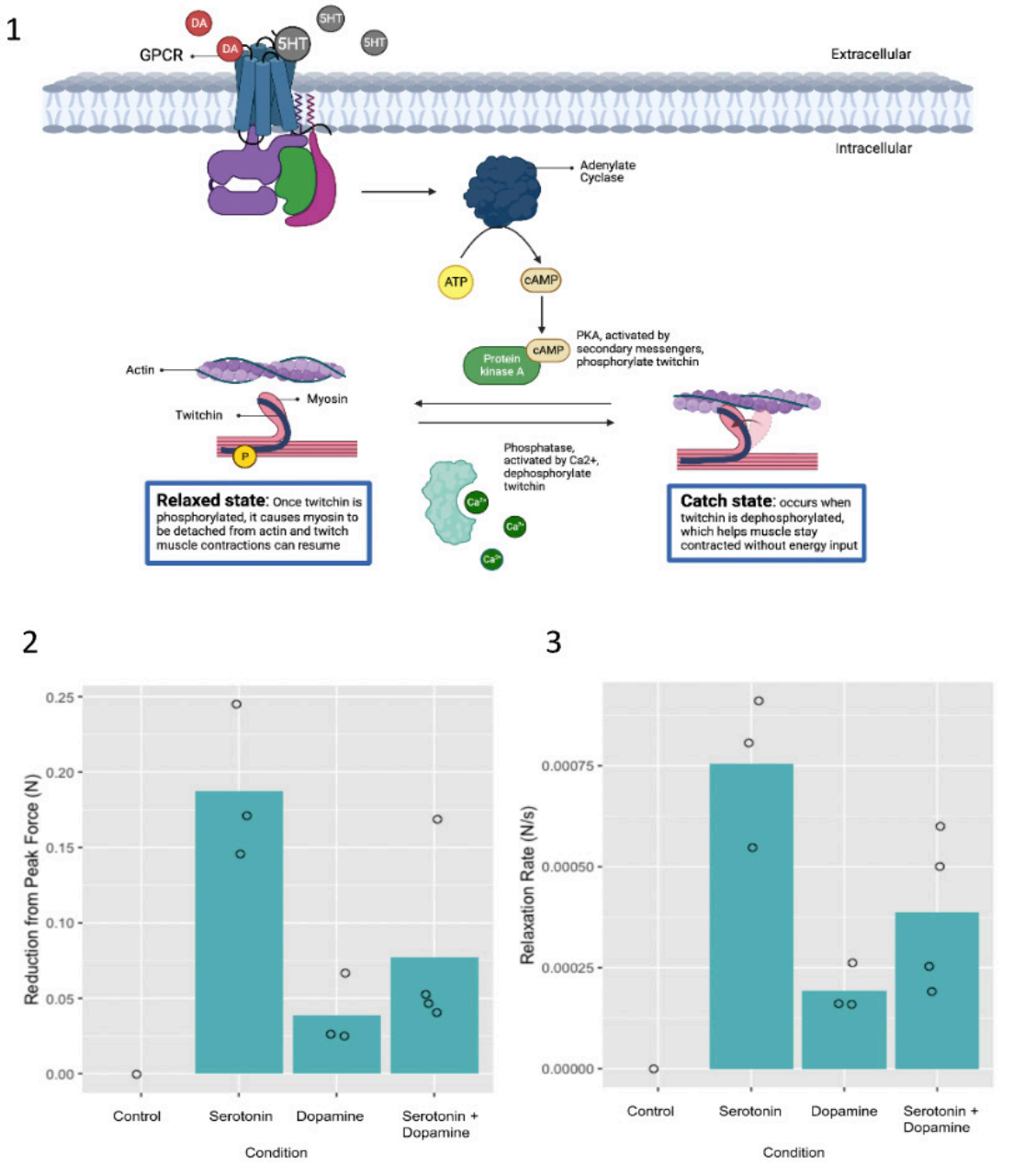

Figure 2: Relaxation response is first measured by the reduction from peak force (N). Column height represents mean and the dots represent the raw data points. When comparing mean reduction from peak force, 5HT-treated muscles (n=3) in catch condition had the greatest reduction from peak force and control (n=1) had the least. Dopamine-treated muscles (n=3) had a low reduction from peak force, but the response was still present. Muscles treated with a mixture of 5HT and dopamine exhibited an intermediate force reduction (between that of 5HT and dopamine).

Figure 3: Relaxation response can also be measured by rate, which was calculated by dividing the reduction from peak force (N) by time it took to relax (secs). Like Figure 1B, column height represents mean and the dots represent the raw data points. Both reduction from peak force and relaxation rate data show similar trends. 5HT-treated muscles in catch condition (n=3) had the fastest mean relaxation rate. Dopamine-treated muscles had a slower mean relaxation rate (n=3), but still greater than the control (n=1). Mussel muscles treated with both 5HT and dopamine have a mean relaxation rate between that of serotonin and dopamine (n=4).

Another limitation for the experiment is that only the amplitude of contraction (after catch is induced) and the change after 5HT and/or DA is added to the mussel were recorded, as well as the time it takes for this relaxation to occur. This yields data in the form of reduction from peak force and relaxation rate. However, it would be beneficial to measure the amplitude of contraction force in order to determine percentage of relaxation due to 5HT and/or DA. A small reduction from peak force could mean that the compound had little effect on catch relaxation, but it could also mean that initial catch contraction achieved a relatively low peak force. This change would not impact the data itself, but it would clarify the discussion of what the results mean.

Furthermore, DA agonist SKF3893 at 10^{-5}M is sufficient to relax catch contractions but does not increase the amount of cAMP in the muscle (Murakami et al., 1986). This presents the possibility that DA has an additional mechanism of hyperpolarization as well as adenylate cyclase activation. Typically, to initiate an action potential, presence of acetylcholine causes sodium channels to open and depolarize the muscle cell. If DA causes hyperpolarization, the cell would require greater influx of Na^+ ions to reach the threshold for action potential. This mechanism could be tested by comparing the concentration of acetylcholine needed to cause catch contraction with and without DA added beforehand. Further research in this direction would elucidate the role of DA in catch contraction as well as catch relaxation in mussels.

Specific Dynamic Action

Metabolic rate is influenced by digestion through a phenomenon called specific dynamic action (SDA), in which metabolic rate increases after food consumption (Rubner, 1902). Metabolic rate is a fundamental physiological parameter that can have significant implications on an animal's survival and fitness outcomes, prompting widespread inquiry of the SDA effect (Burton et al., 2011). Previous research has established the presence of SDA across a wide range of organisms, suggesting it is a common physiological phenomenon (Jobling & Davies, 1978). However, there are still gaps in understanding regarding the mechanisms and magnitude of SDA across different species and contexts. For example, recent studies have suggested that the magnitude of SDA in fish may increase in duration but decrease in magnitude as temperature decreases (Tirsgaad, 2015). Understanding these factors and their effects on SDA can provide insights into the ecological and evolutionary significance of this phenomenon, as well as inform practical applications such as animal feeding and husbandry practices in aquaculture and agriculture.

To study the scope of SDA, we designed an experiment to compare the metabolic rate of fed and fasted zebrafish populations We hypothesized that 1) metabolic rate would be higher in the exercise condition due to additional muscle energy expenditures and 2) metabolic rate would be higher in the fed condition due to the SDA effect. By testing our hypotheses about the effects of exercise and feeding on metabolic rate, we aimed to contribute to our understanding of the underlying mechanisms of SDA and its potential implications in energy expenditures.

Understanding the impact of these factors on metabolic rate is crucial for predicting energy expenditures and ultimately, survival and fitness outcomes.

METHODS

To study the scope of specific dynamic action (SDA), an experiment was designed to compare the metabolic rate of fed and fasted zebrafish populations. The experiment was conducted using a single previously fasting zebrafish, which was acclimated to the flask's environment for 15 minutes before the start of the experiment. Following the acclimation period, the zebrafish was either fed 10mg of fish food (Fig. 1) or fasted for 30 minutes. The use of a standardized fish food ensured that the nutritional content of the food was consistent between individuals and that any observed differences in the SDA response could be attributed to other factors, such as activity level or the fed versus fasted state.

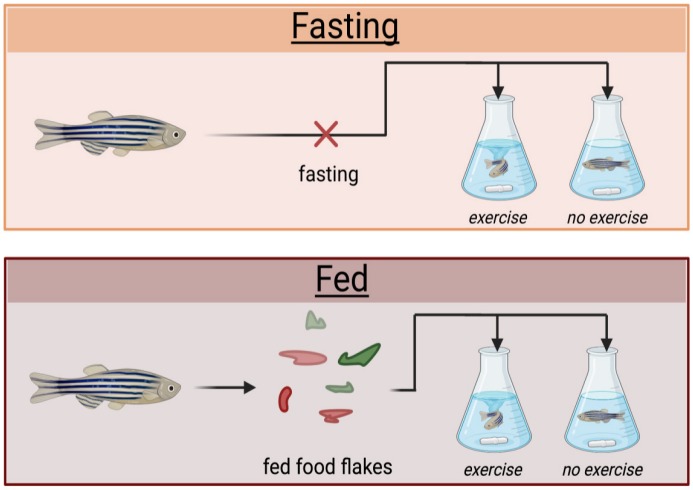

Figure 4. The experimental design, adapted from the design by Trueblood, featured both a fasted (orange) and fed (dark red) population (2015). The populations were then subjected to either an exercise or no exercise condition. In the exercise condition, the zebrafish were active due to the creation of a current by a magnetic stir bar. In the no exercise condition, the zebrafish were not subjected to any external stimuli.

After the feeding or fasting period, the metabolic rate of the zebrafish was measured using an oxygen probe for 15-30 minutes, as described by Trueblood (2015). For both populations, a condition of exercise and no exercise was implemented to measure the effect of physical activity on metabolic rate and SDA. In the exercise condition, the zebrafish were subjected to physical activity by creating a current in the flask using a magnetic stir bar. The no exercise condition, on the other hand, involved no external stimuli, allowing the zebrafish to remain at rest. To control for the possible magnetic field influence of the stir plate, all flask environments were placed on top of the magnetic stir plate with a stir rod inside, even if it was inactive. In all trials, the casper strain of zebrafish was used to control for genetic variation. All zebrafish were held in the same tank environment prior to the initiation of the experiment and fasted for 12 hours to reduce discrepancies.

RESULTS

A total of eleven trials were conducted. In both the no exercise and exercise condition (Fig. 2), the fasted fish exhibited a higher mean mass-specific metabolic rate (MSMR). Across all conditions, the no exercise while fasting condition exhibited the highest mean MSMR, 0.0011 mL O2/ s*g. In contrast, the exercise while fed condition exhibited the lowest mean MSMR, 0.00022 mL O2/ s*g. The other two conditions, no exercise while fed and exercise while fasting, had respective mean MSMRs of 0.0070 and 0.0071 mL O2/ s*g. Unexpectedly, the exercise groups did not exhibit a higher mean MSMR in comparison to the no exercise groups. The range of values across all groups was 0.002 to 0.0014 mL O2/ s*g, suggesting a large variance between the data points.

In terms of distribution, the fasted exercise fish had the widest range of data values, 0.00022 to 0.0014 mL O2/ s*g. In contrast, the fed exercise fish exhibited the tightest distribution and range, 0.00011 to 0.00027 mL O2/ s*g. It is important to note, however, that the exercise while fasting fish had a significant outlier at 0.0014 mL O2/ s*g. It was significantly different from the rest of the group, whose data points otherwise ranged from 0.002 to 0.006 mL O2/ s*g. This likely contributed to the group's wide distribution and larger mean (Fig. 1B). This data point was also the maximum MSMR measured across all groups.

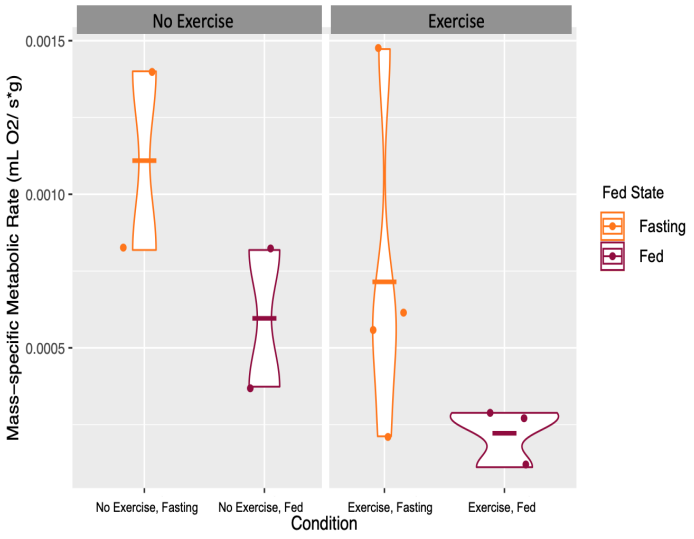

Figure 5. Specific dynamic action (SDA) was not observed in this experimental design, as fasting fish exhibited a higher mass-specific metabolic rate (mL O2/ s*g). The fasted fish exhibited a higher mean mass-specific metabolic rate (mL O2/ s*g), as illustrated by the horizontal line, in both the no exercise and exercise condition. In terms of distribution, the fasted exercise

fish had the widest distribution of data values, as shown by the long length of the violin plot. In contrast, the fed exercise fish exhibited the tightest distribution.

DISCUSSION

We hypothesized that the metabolic rate would be higher in the fed condition due to the SDA effect. However, the fasted fish exhibited a higher mean MSMR (Fig. 1B) in both exercise conditions. Current literature suggests that metabolic rate should increase due to the additional energy expenditures of digestion associated with SDA (Brown & Cameron, 1991). Similar investigation in plaice fish found an increase of metabolic rate, ranging from 141.8% to 191.6%, in the following 2 days after feeding (Jobling & Davies, 1978). However, the results in zebrafish contradict previous findings. The fed zebrafish population exhibited a lower mean MSMR in both exercise conditions, challenging our proposed hypothesis. Because fed fish did not exhibit a higher metabolic rate as expected, these results suggest that the SDA effect did not occur.

Analytically, the absence of SDA is unexpected. Physiologically, the energetic costs associated with the absorptive processes of digestion should result in a noticeable in increase in metabolic rate (Hailey, 1987). Meal heating, enzyme secretion, protein catabolism, and intestinal absorption have been recorded to contribute to SDA metabolic surges, as demonstrated in pit viper experimentation (Tsai et al., 2008). Similar digestive processes occur in zebrafish digestion, drawing doubt to the lack of observed metabolic rate increase. However, a few explanations may justify the absence of SDA. Firstly, it is possible that SDA occurs after the 15-30 min. window measured, due to the latency of digestion. Previous investigation with goats has recorded a delay in which digestive processes take place, possibly due to the necessity for enzyme production and associative protein scaling (Magee, 1924). As such, SDA may still have occurred in the zebrafish populations, but beyond the 15-30 min. window. Future experimentation should include a longer measurement window to ameliorate this limitation.

Moreover, acute stress from a new environment may have limited digestive processes and thereby minimized the SDA effect. Previous investigation has noted that the transport and variance in holding containers associated with laboratory conditions can increase zebrafish cortisol (Dhansari et al., 2013). Increased cortisol is known to stimulate fat and carbohydrate metabolism to increase glucose availability, as shown in prior human injection (Brillon et al., 1995). Thus, acute transfer stress could have increased metabolic rate across all groups, potentially reducing the magnitude of SDA's effect. To ameliorate this phenomenon, a greater acclimation period should be implemented to reduce acute stress, which may prevent energy allocation to digestive processes and thus reduce the magnitude of SDA.

Beyond the short measurement window and acclimation period, the experimental design may have been further limited. Meals with higher protein compositions have been recorded to have higher SDA effects in plaice fish, likely due to additional protein catabolism (Jobling & Davies, 1978). As such, the fish food composition may have been lacking in protein, which would limit the magnitude of SDA. To fix this, future experiments should use high-protein compositions to increase SDA presence and magnitude. Additionally, the use of Parafilm to cover the flask may have led to an incomplete seal, allowing gaseous oxygen to diffuse into the water. The diffusion of oxygen would increase oxygen readings, lowering the measured metabolic rate and thereby disguising any SDA effect. The wide distribution of data values for each condition, as shown through the long lengths of the violin plots (Fig. 1B), suggests that the readings were not consistent, and this insufficient seal may have occurred. Future experiments should use a modified silicone stopper, which would create a better seal and tighter distribution of data values.

Furthermore, additional inquiry should investigate the potential variance of SDA across different life stages. Metabolism is known to fluctuate across an individual's lifespan, notably declining as humans age (Milward et al., 1997). Intestinal efficiency also declines with age due to changes in gut microbiota and other factors (Coudray et al, 2006). As such, future experimentation should investigate if increased age, along with declines in intestinal efficiency, reduces the SDA response. Overall, while the results did not

record an SDA effect, the data suggests SDA may be influenced by more environmental conditions that previously believed.

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FIGURES

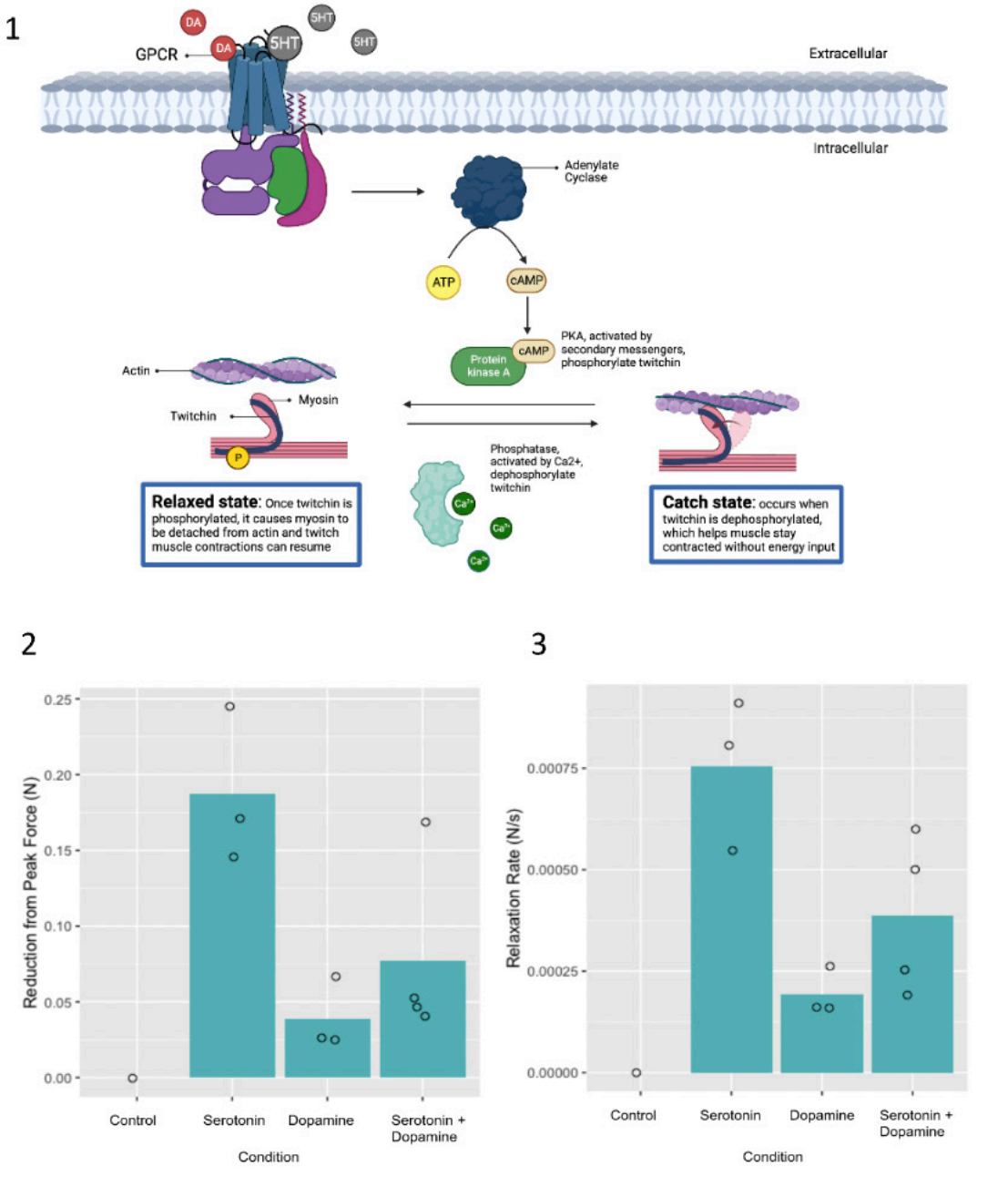


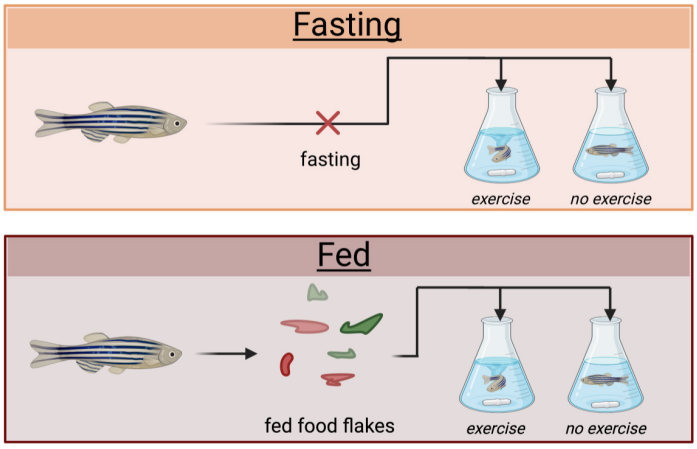
Figure 1: Dopamine and serotonin both activate the signaling cascade to phosphorylate twitchin and relax catch contractions in mussels – 5HT's effect on relaxation response is stronger than DA's, and relaxation response to a combination of 5HT and DA is greater than that of DA alone, but less than that of 5HT alone.

A) Serotonin, and dopamine (not pictured), are extracellular signaling molecules that bind to G-protein coupled receptors (GPCR). GPCRs set off an intracellular signaling cascade that activates adenylate cyclase, an enzyme that converts ATP to cAMP. cAMP is a secondary messenger that activates protein kinase A (PKA), which phosphorylates twitchin. Once phosphorylated, twitchin releases actin and myosin and relaxes the catch contraction. To begin a catch contraction, a phosphatase activated by Ca^{2+} dephosphorylates twitchin, which allows twitchin to bind myosin and actin. Adapted from Funabara et al.,

2007.

B) Relaxation response is first measured by the reduction from peak force (N). Column height represents mean and the dots represent the raw data points. When comparing mean reduction from peak force, 5HT-treated muscles (n=3) in catch condition had the greatest reduction from peak force and control (n=1) had the least. Dopamine-treated muscles (n=3) had a low reduction from peak force, but the response was still present. Muscles treated with a mixture of 5HT and dopamine exhibited an intermediate force reduction (between that of 5HT and dopamine).

C) Relaxation response can also be measured by rate, which was calculated by dividing the reduction from peak force (N) by time it took to relax (secs). Like Figure 1B, column height represents mean and the dots represent the raw data points. Both reduction from peak force and relaxation rate data show similar trends. 5HT-treated muscles in catch condition (n=3) had the fastest mean relaxation rate. Dopamine-treated muscles had a slower mean relaxation rate (n=3), but still greater than the control (n=1). Mussel muscles treated with both 5HT and dopamine have a mean relaxation rate between that of serotonin and dopamine (n=4).



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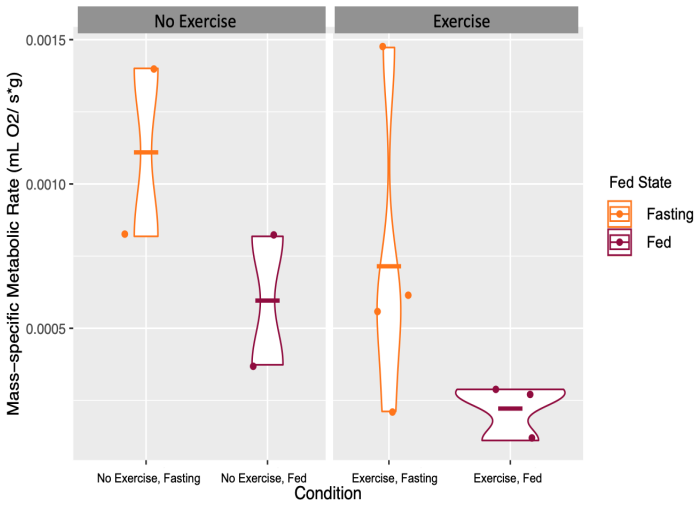

Figure 5. Specific dynamic action (SDA) was not observed in this experimental design, as fasting fish exhibited a higher mass-specific metabolic rate (mL O2/ s*g). The fasted fish exhibited a higher mean mass-specific metabolic rate (mL O2/ s*g), as illustrated by the horizontal line, in both the no exercise and exercise condition. In terms of distribution, the fasted exercise fish had the widest distribution of data values, as shown by the long length of the violin plot. In contrast, the fed exercise fish exhibited the tightest distribution.