Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Pharmacology and Toxicology

ISSN 1816-496X
DOI: 10.3923/jpt.2025.1.20

Research Article
Physiological Effects of Sodium Selenite on Behavior, Cardiac, Neural and Synaptic Functions in *Drosophila*, Crayfish and Crab

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Abstract

Background and Objective: The element selenium is part of the amino acid selenocysteine and aids in the function of selenoproteins. These proteins promote cellular function and the immune system in mammals. The inorganic form of selenium is sodium selenite and is used in clinical therapies to inhibit ferroptosis. The objective of this study is to investigate the physiological effects of sodium selenite on behavior, cardiac function, neural activity and synaptic transmission in *Drosophila*, crayfish and crabs. This research aims to evaluate the impact of sodium selenite on these biological systems to enhance the understanding of its potential benefits or toxicity across different model organisms. **Materials and Methods:** This investigation examined the effects of acute sodium selenite exposure in relatively high concentrations (1 and 5 mM) on sensory nerves of the marine crab (*Callinectes sapidus*); stretch-activated channels (SACs) in the muscle receptor organ of a freshwater crayfish species (*Procambarus clarkii*); synaptic transmission at the neuromuscular junctions of crayfish and larval *Drosophila melanogaster* and the myogenic heart of larval *Drosophila*. Electrophysiological recordings were obtained with intracellular and extracellular recordings. Statistical analysis included Shapiro-Wilk or Kruskal-Wallis ANOVA to assess normality, with t-tests, paired t-tests or Wilcoxon Signed-Rank tests used for significance. Weibull regression modeled survival curves, while paired t-tests or Wilcoxon tests compared behaviors before and during selenium selenite exposure. A significance level of 0.05 was applied in all analyses. **Results:** Systemic exposure of sodium selenite in crayfish hemolymph resulted in organism death after 2 hrs. The effects on the development and survival of *Drosophila* with dietary ingestion revealed that larvae died in 24 hrs and adults in 3 days with food laced with 1 mM sodium selenite. Exposure to 1 mM sodium selenite killed *Drosophila* larvae and adults in 24 hrs and a few hours for injected crayfish. Synaptic transmission at the *Drosophila* neuromuscular junction was acutely depressed with exposure but could recover with removal. **Conclusion:** This indicates that the effects vary between acute exposure to tissue and chronic exposure of 1 or 2 days in intact animals. Acute selenium overexposure largely disrupted neurological and cardiac function, behavior, development and survival of *Drosophila* and crayfish.

Key words: Crayfish, crustacean, development, *Drosophila*, larvae, neuron, neuromuscular, selenium, sensory

Citation: Brock, K.E., J.E. Gard, E.R. Elliott, A.C. Taul and J.B. Nadolski *et al.*, 2025. Physiological effects of sodium selenite on behavior, cardiac, neural and synaptic functions in *Drosophila*, crayfish and crab. J. Pharmacol. Toxicol., 20: 1-20.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Selenium is a vital trace element for both humans and animals. It is crucially involved in several different roles in the human body, including antioxidant defense. Selenium is a key component of selenoproteins, essential for enzyme function associated with free radicals and oxidative stress protection. The antioxidant properties of selenium protect cells from damage and aid the body's repair processes. For this reason, the use of selenium supplements is curtailed during radiation therapy but bolstered when an individual is at risk of unintentional radiation exposure[1]. Selenium is necessary for the proper functioning of the thyroid gland[2], as it aids in the conversion of thyroxine (T4) into its active form, triiodothyronine (T3)[3], while adequate selenium levels are associated with improved cognitive function and may help reduce the risk of cognitive decline with aging[4]. Selenium has been examined for its antiviral effects and links to cardiovascular health and cancer prevention[2]. Due to its essential roles in antioxidant defense and thyroid function, selenium deficiency can lead to serious health issues, such as white muscle disease in animals and Keshan disease, a type of cardiomyopathy, in humans[5]. However, while essential in small quantities, selenium can become toxic at higher levels.

Selenium can be found naturally in soil, water sources and plants. Plants first obtain selenium through the absorption of soil and water; then, as insects and other animals consume these plants, they subsequently ingest trace amounts of selenium[6]. Selenium is ingested in various manners and may be converted to different chemical forms at the cellular level.

Selenium can bioaccumulate in various organisms, entering human systems both directly and indirectly. Indirect consumption occurs due to selenium being added to animal diets or plant nutrients[7]. Direct consumption of selenium includes dietary supplements and skincare products, such as shampoos, that contain selenium additives[8]. Additionally, selenium has been extensively researched for its potential use as an insecticide[9]. Volcanic activity as well as human activities like mining, agriculture and industrial processes can release excess selenium into the environment[6], where it acts as a pollutant[9].

Both acute and long-term exposure to selenium can result in selenium toxicity[10]. High concentrations of selenium yield clinical symptoms in humans such as gastrointestinal upset, hair loss, blotchy white nails and mild nerve damage, as well as nausea, vomiting, fatigue and dizziness[8,10]. Acute exposure occurs due to a rapid increase in reactive oxygen species accompanied by stress-induced damage[11]. Tachycardia and fluctuations in blood pressure also indicate potential cardiovascular effects[12]. There are currently no known treatments or antidotes for selenium overdose; it is merely recommended that exposure be restricted and the symptoms treated[10]. This knowledge gap underscores the critical need for continued research into mechanistic actions, effective interventions and preventive measures.

Previous studies have shown that selenium exposure severely impacts insects, with high levels disrupting development and causing significant declines in fertility across various species[13]. Exposure to selenium in food significantly impairs growth in insects and acute exposure to high concentrations severely disrupts their learning and memory functions[13]. Crustaceans are also greatly affected by selenium toxicity, which leads to profound cellular degeneration, necrosis and apoptosis in the hepatopancreas, thus disrupting digestive enzyme production and compromising nutrient absorption (see reviews by researchers[14-18]).

Current studies utilize sodium selenite, the water- and saline-soluble form of selenium, to investigate the acute, direct effects of selenium on physiological functions. This report focuses on how selenium affects neuronal and cardiac function. The use of sensory neurons from crayfish and crab models allows for a direct assessment of selenium's neuronal effects and electrical transmission without synaptic input. The crayfish heart, meanwhile, is neurogenic and relies on neural input to regulate the rate of contractions. Monitoring cardiac function in an intact crayfish allows for the simultaneous assessment of multiple physiological functions, as the model's heart rate is highly responsive to sensory stimuli and is thus a useful indicator of how these stimuli (i.e., physical touch or vibration in the water) affect cardiac function[19-21]. A forceful tap on the telson results in cardiac function pausing for a few msec, followed by a rapid increase in heartbeat rate. This process is driven by sensory input to the CNS and likely integrates with the autonomic nervous system in the crayfish model[21]. Additionally, the survival of the intact crayfish can be monitored following the systemic injection of compounds. Glutamatergic synapses at the crayfish and larval *Drosophila* isolated neuromuscular junctions (NMJs) also constitute a fitting model for investigation into synaptic transmission, as the muscles do not produce action potentials and the excitatory junction potentials are graded[22-24].

Larval *Drosophila* also offers *in situ* access to a myogenic pacing heart absent of neural innervation and can be maintained at length in physiological saline devoid of hormones and other cardiac modulators[25,26]. *Drosophila* has been used to examine the effects of compounds through dietary consumption; specifically, studying development, survival during development and adult survival after exposure has provided valuable insights into the effects of these compound[27-29]. *Drosophila's* genetic amenability

allows for relatively easy examination of ion transport systems and cellular processes through gene alteration as compared to other models. Altogether, developing an understanding of how essential metals like selenium are absorbed, transported and stored in *Drosophila* can offer valuable insights into these processes in other animals[30]. Indeed, *Drosophila*, as a model, has played a vital role in advancing the understanding of biological systems and furthering clinical research[31-36]; thus, this initial investigation into selenium's effects on *Drosophila* could serve as the foundation for future studies to expand our understanding of the element's impact across various other species. Furthermore, these studies can help develop more comprehensive models to explore selenium's broader implications for health and physiology in diverse organisms.

This report was intended to survey the acute effects of selenium on physiological functions using various invertebrate models, as well as the chronic effects (on development and survival) in *Drosophila melanogaster*. Further studies could investigate the long-term effects of organic compounds containing selenium in diets, such as selenomethionine and selenocysteine, though this is beyond the scope of the current study.

MATERIALS AND METHODS

Study area: This research was conducted at the University of Kentucky in Lexington, Kentucky, USA, between May, 2024 and November, 2024.

Animals: *Drosophila melanogaster* Canton S (CS) flies were used in all behavioral and physiological assays. This strain was originally obtained from Bloomington *Drosophila* Stock Center (BDSC) and has remained isogenic in the lab for several years. All animals were housed in vials partially filled with a cornmeal-agar-dextrose-yeast medium. Blue crabs (*C. sapidus*) were delivered from a distribution center in Atlanta, GA, USA and then purchased from a local supermarket in Lexington, KY, USA, whereupon they were maintained in a seawater aquarium for several days prior to use to assess their health. All crabs were adults ranging from 10-15 cm in carapace width (from point to point). They were alive and very active upon autotomizing a leg for experimentation. Red Swamp Crayfish (*Procambarus clarkii*) were delivered from a distribution center in Atlanta, GA, USA and then purchased from a local supermarket in Lexington, Kentucky, USA. Midsized crayfish measuring 6-10 cm in body length and 12.5-25 g in body weight were used. Each animal was housed individually in a plastic container with aerated water (20-21°C). Dry fish food was exchanged weekly[28].

To determine reproducibility, senior-level university students in a neurophysiology course repeated some of these experiments while being blinded to the results obtained previously[37,38]. Independent data sets were gathered by different investigators and are presented separately for comparison. This extra step of examining reproducibility was conducted as some results were inconclusive and featured a great degree of variability. The experiments were replicated independently to determine whether this was the case for other researchers as well.

Survival and developmental studies of *Drosophila melanogaster*: To address the effects of sodium selenite on life cycle alterations, the time from 1st instar larva to pupation was measured when exposed to sodium selenite (1 mM) through tainted food. Ten plastic vials, each with ten 1st instar larvae, were given 1 g of corn meal food mixed with 1 mL of 2 mM sodium selenite. Another set of 10 vials, with 10 larvae in each, were given 1 g of food mixed with 1 mL of deionized water for control. Within 12 hrs of emerging from eggs, all larvae were placed on the food located at the bottom of the vials. Cotton lids were used to allow for an aerated and moist environment at 21°C. The larvae were then left to develop into a pupa. Each day, the number of pupae was counted for each condition. After 7 days in vials that did not develop pupa, the food was removed and the contents were examined for dead larvae.

Adults were collected within 2-3 days after eclosion from pupa to assess for survival. Ten adults were then placed in vials containing 1 g of food mixed with 1 mL of 2 mM sodium selenite. An additional ten adults were exposed to 1 g of food mixed with 1 mL of deionized water for control. Conditions were replicated for ten trials and survival was monitored daily.

Larval behavioral assays

Body wall movements and mouth hook movements: Locomotion of larvae was assessed by recording body wall movements (BWM). Twenty late 2nd instar larvae were placed in 1 g of food mixed with 1 mL of 1 mM sodium selenite. Another twenty late 2nd instar larvae were placed in 1 g of food mixed with 1 mL of deionized water for control. This was also repeated to provide food tainted with 0.1 mM sodium selenite. The 1 g of food was assumed to be equivalent to 1 mL for dilution of the stock solutions. After 24 hrs, the body wall contractions were monitored. The larvae were placed on filter paper (Whatman #3) within plastic Petri dishes (about 8.5-9 cm diameter) saturated with pure apple juice to promote locomotion. Using a dissecting microscope, contractions per min were visually counted.

After each larva was monitored for body wall movements, it was transferred to a dish for monitoring mouth hook movements (MHM). The small, smooth bottom Petri dish (5.5 cm diameter) contained a yeast solution (a few dried yeast granules mixed with water) to promote feeding without body wall locomotion. The solution level was kept low to allow protrusion of the larval spiracles from the solution. The number of MHM was counted visually with the use of a dissecting microscope (adjustable zoom 0.67-4.5; World Precision Instrument, Florida, USA). The BWM and MHM were counted in a lightly illuminated environment at room temperature (21-22°C).

Beating rate of the larval heart in *Drosophila melanogaster*: A detailed description, in video format, of a method by which to expose the larval heart in early 3rd instars is provided in Stewart *et al.*[39]. In brief, the larvae were dissected ventrally and pinned at the four corners to expose the heart tube. The preparation was then bathed in physiological saline[24]. A modified HL3 saline was used to maintain the *in situ* hearts and body wall muscles (NaCl 70 mM, KCl 5 mM, $MgCl_2.6H_2O$ 20 mM, $NaHCO_3$ 10 mM, Trehalose 5 mM, sucrose 115 mM, BES 25 mM and $CaCl_2.2H_2O$ 1 mM, pH 7.1[39]). Sodium selenite was added to the saline to produce a concentration of 1 or 5 mM. The pH of the saline was maintained at 7.1.

The heart rate was counted visually via a dissecting microscope (adjustable zoom 0.67-4.5; World Precision Instrument, Florida, USA) and has been presented in beats per min for graphical analysis. Measures were taken in the caudal region of the heart tube (Fig. 1). Rate was measured during the initial saline exposure and after an exchange of the bathing solution to saline containing sodium selenite; then, the preparation was flushed twice with fresh saline and the beats were counted again. For each medium exchange, the heart rate was measured as soon as the new solution was added and different preparations were used for each concentration of sodium selenite. The rates were counted in a lightly illuminated environment at room temperature (21-22°C).

Cardiac responses and survival of crayfish: Heart rates in crayfish were obtained through an impedance measure. The recording procedure has been described in textual and video format[40] and has been used for the injection of iron into the hemolymph of the crayfish[36]. Insulated stainless steel recording wires (diameter 0.005/0.008 inches with coating; A-M Systems, Carlsberg, WA) were used and the insulation was burned off the ends with a flame to provide a good connection to the recording devices. Small holes were punctured through the cuticle of a thickness approximate to that of these wires to minimize hemolymph loss and maximize the probability that the wires remain in place during fixation; the insulated steel wires were then threaded through the holes and into the carapace, spanning the heart, to facilitate an accurate impedance measure (UFI, model 2991)[19].

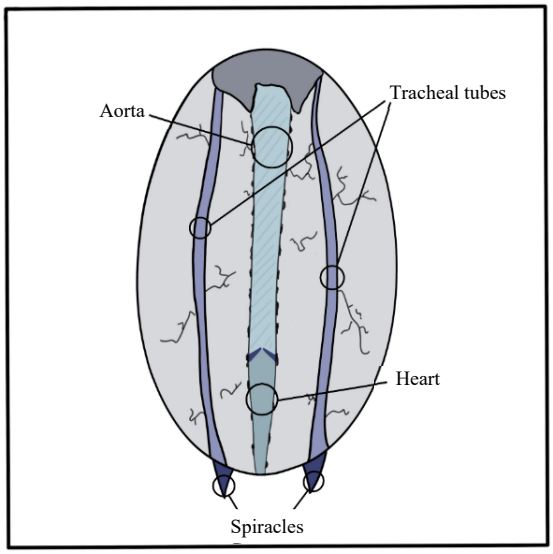

Fig. 1: Filleted larval preparation for measurement of the heart tube's beating rate before and during exposure to sodium selenite
Heart rates were counted by manual inspection through a dissecting microscope before and after switching to the compound of interest. The counts were obtained from the caudal end of the preparation and near the point where the two tracheal tubes bifurcate

To eliminate the risk of damaging the heart, special attention was paid to inserting only a short portion of wire (1-2 mm). After placing the wire in the optimal position, fixation was ensured via a small drop of glue (cyanoacrylate ester) and accelerator (HobbyTown, Lexington, Kentucky, USA). The impedance detector, measuring the dynamic resistance between the two wires, was linked to a PowerLab/4SP interface (AD Instruments, Australia). The acquisition rate was set at 1 kHz. Calculation of the heart rate was accomplished by direct counts of each beat over 1 min intervals and reported as beats per min (BPM)[36].

Either normal saline or saline containing sodium selenite was injected into the crayfish's abdomen by passing a needle through the articulating membrane on the ventral side near the lateral aspect. This prevents damage to the underlying muscles but allows direct and rapid mixing with the hemolymph. Control experiments using saline were conducted to account for any excitation of the animal caused by handling and injection alone. Systemic levels of sodium selenite were calculated based on dilution in the hemolymph from a stock concentration of 10 mM, using an estimation of each organism's hemolymph amount based on the assumption that 30% of an animal's weight is hemolymph[41,42]. The total volume had to be established to determine the appropriate volume of stock that should be injected. The physiological saline was comprised of the following modified Van Harreveld's solution: In mmol/L: 205 NaCl; 5.3 KCl; 13.5 $CaCl_2.2H_2O$; 2.45 $MgCl_2.6H_2O$; 10 Glucose; 0.5 HEPES adjusted to pH 7.4. Since the animals have an open circulatory system, the compounds are carried toward the heart, where they bathe the cardiac ganglion and muscle. Experiments were carried out using six animals for saline and six for sodium selenite.

To assess whether the neurogenic heart was responding to sensory stimuli, the central nervous system was activated and the neural input to the cardiac ganglion was altered with forceful tapping of the crayfish telson with a glass rod to elicit a tail-flip response. The recording was maintained for 20 min after the tap, followed by saline or sodium selenite injections. After an additional 20 min, the telson was forcibly tapped again to note the stimulus' effects on the heart rate. The effects of the telson tap were monitored periodically over 24 to 48 hrs after the injection (Fig. 2). This injection protocol also provided an assessment of the injections of sodium selenite on the survival of the crayfish over 14 days.

Crayfish muscle receptor organ: The dissection procedure is described in video format[43] and involves the same experimental procedure as previously described when assessing the effects of muscle receptor organ (MRO) exposure to iron[36]. The muscle receptor neurons of the crayfish abdominal proprioceptor are fully exposed to the bathing saline and produce spikes upon manipulation of the abdominal joint that can be measured with extracellular recording. As the segmental nerve only measures two neurons, one sensitive to dynamic movement and one sensitive to static movement, fewer variations in spike amplitudes are recorded. The sensory neurons have their endings embedded within a single associated muscle fiber for each neuron. The muscle fibers span the joint in which they monitor and reside directly under the dorsal cuticle.

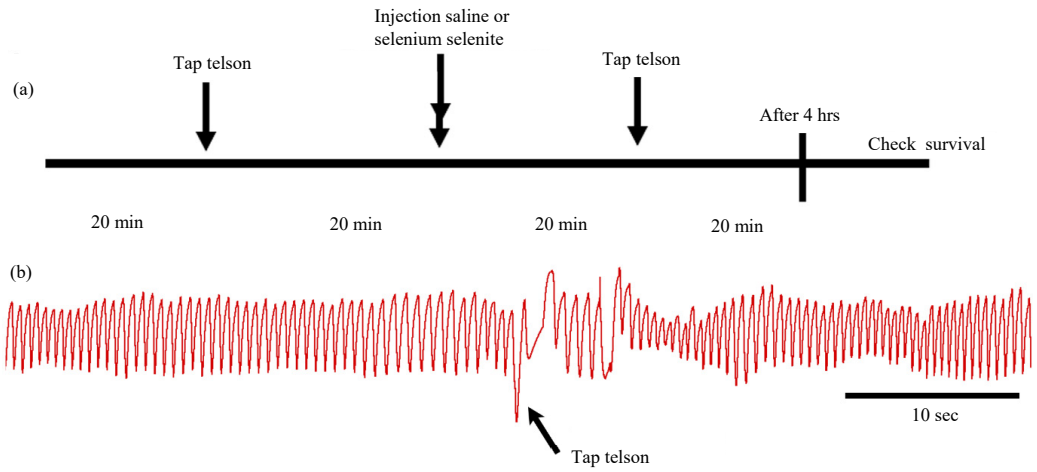

Fig. 2(a-b): Experimental paradigm for monitoring crayfish cardiac function

Rate was determined before and after a telson tap, as well as before and after injection of either saline or sodium selenite. The telson tap, monitoring of the response to the tail flip and examination of the effects on heart rate were repeated after 20 min of injection and after 24 and 48 hrs

As the joint is moved rapidly (across 1 sec) to a set position and then held for at least 9 sec more, both types of neurons can be monitored. Abdominal joints not involved in the recordings were pinned in a Sylgard-lined dish and covered with crayfish saline. For the recording of extracellular signals from the cut nerves, the MRO nerve was then exposed and pulled into a suction electrode made from a glass pipette fitted with a plastic tip (details provided in Baierlein *et al.*[44]). A P-15 amplifier (Grass Instruments) was used in conjunction with a PowerLab/4s A/D converter and Lab Chart 7 software (ADI Instruments, Colorado Springs, Co., USA) to obtain the signals for recording on a computer at a 20 kHz sampling rate. The neural activity was easily distinguished from the baseline noise present when the MRO was relaxed and stationary.

During the experiment, the joint of interest was moved over a 1 sec time frame, held in position for at least 9 sec, then moved back to the starting position to rest (10 sec) before the next displacement was performed. An insect dissecting pin was used as a reference to ensure consistency in the maximum displacement range and each displacement was marked on the computer recording file. The movements were repeated thrice in each medium to represent three trials, with identical rates and degrees of movement. This was conducted while the preparation was exposed to saline only, after the solution had been swapped out for one containing the compound sodium selenite (5 mM), after the preparation had incubated for 10 min and then again upon two rinses of the preparation (to remove the sodium selenite) and return to fresh saline.

The number of spikes recorded over 10 sec was used for the analysis of each trial in each condition; additionally, the average number of spikes for each of the three displacements in each condition was also determined for graphical purposes. A percent change among the preparations was also used for analysis to normalize differences among preparations in the number of spikes initially obtained in saline. A paired t-test (if the Shapiro-Wilk test passed) or a Wilcoxon Signed Rank test was used to compare the differences in responses before and during exposure to sodium selenite.

Crab chordotonal organ: The dissection procedures and electrophysiological measures used here were similar to those thoroughly described in text and video format by Majeed *et al.*[45]. This is the same experimental procedure as has been previously described for assessing the effects of propodite-dactylopodite organ exposure to zinc[37]. In brief, the animal was induced to autotomize the first or second walking leg by lightly pinching the base of the leg with pliers. The propodite-dactylopodite (PD) chordotonal organ spans the last segment of the leg and was cut at a window of the cuticle on both sides of the leg (in the propodite segment), whereupon the leg was pinned in a

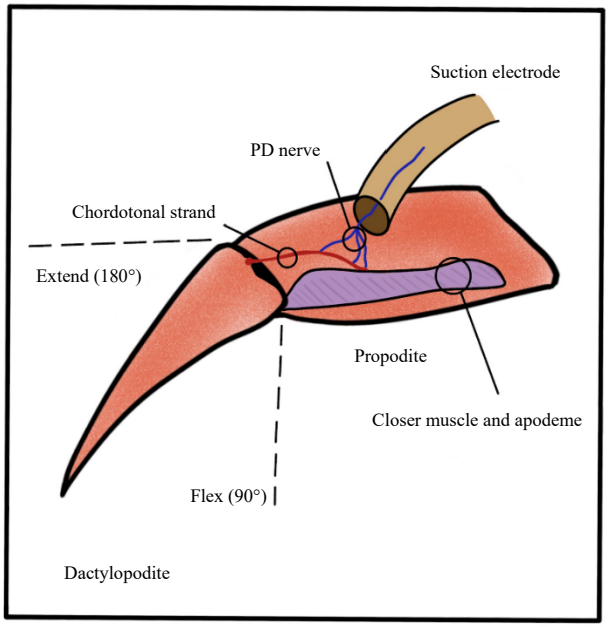

Fig. 3: Crab leg exposure

First or second walking leg of the crab was used, from which the PD organ and associated nerve were exposed. The joint was initially flexed at 90° and then extended out to 180° within 1 sec, whereupon it was held for at least 9 more sec

Sylgard-lined dish and covered with crab saline. The PD nerve was then exposed and pulled into a suction electrode for recording (Fig. 3). During the experiment, the dactyl was moved from a flexed position to an open position during a 1 sec time frame, held for at least 9 more sec and then moved back to the starting position. An insect dissecting pin was used as a reference for the maximum displacement range and each displacement was marked on the computer recording file. The joint was moved back to its starting position and allowed to rest for 10 sec after each displacement before the next displacement was given. The crab saline used during recordings of the sensory nerves consisted of (in mM): 470 NaCl, 7.9 KCl,15.0 $CaCl_2.2H_2O$, 6.98 $MgCl_2.6H_2O$, 11.0 dextrose, 5 HEPES acid and 5 HEPES base adjusted to pH 7.4. Sodium selenite (5 mM) was added to this saline to examine the effects on neural responses. The number of spikes recorded over the first 10 sec, from the start of the joint displacement, was used as an index of the neural activity.

The same paradigm used for the crayfish MRO was applied to the crab PD organ. The movements were repeated thrice in each condition to represent three trials, with identical rates and degrees of movement; this was conducted while the preparation was exposed to saline only, while exposed to sodium selenite (5 mM), after 10 min incubation time and after a double-wash to remove the sodium selenite and the subsequent return to fresh saline.

Synaptic transmission at crayfish and larval *Drosophila* neuromuscular junctions: The dissection procedures and electrophysiological measures used here were similar to those thoroughly described in text and video format[46]; this is also the same experimental procedure described previously to assess the effects of manganese exposure at crayfish and larval *Drosophila* NMJs[37]. In brief, the excitatory neuron was isolated from the inhibitor neuron and then stimulated in the meropodite segment. The stimulation paradigm consisted of providing a train of pulses at 60 Hz with 10 sec between trains. Responses were recorded with an AxoClamp 2B (Axon Instruments, USA), converted with a PowerLab, 4SP (ADInstruments, USA) and analyzed with LabChart 7.0 (ADInstruments, Colorado Springs, CO, USA) on a computer at a 20 kHz sampling rate. Dissected preparations were maintained in crayfish saline as stated above for the crayfish MRO preparation. Sodium selenite (5 mM) was added directly to the saline to examine the effects on synaptic transmission.

The last evoked excitatory junction potential (EJP) in the train was used as an index for the effect of sodium selenite (5 mM) on the amplitude of the EJPs. The amplitude was measured from the base of the last EJP to its peak. The preparations were recorded for at least 250 sec in saline, 500 sec while exposed to sodium selenite (5 mM) and another 250 sec after flushing the bath with fresh saline. The stimulus trains were delivered every 10 sec to provide enough time (~8.3 min) for the acute effects of sodium selenite to be assessed, as previously done for Zn^{2+}, Mn^{2+} and Fe^{3+} [26-28,36,37].

Third-instar larval *D. melanogaster* was dissected in physiological saline, the composition of which has been described above for the larval *Drosophila* cardiac measures. The segmental nerves were cut, sucked into a suction electrode filled with saline and stimulated at 0.5 Hz (S88 Stimulator, Astro-Med, Inc., Grass Co. West Warwick, RI, USA). To monitor the transmembrane potentials of the body wall muscle (m6) of 3rd instar larvae, a sharp intracellular electrode (30 to 40 M resistance) filled with 3M KCl impaled the fiber. An Axoclamp 2B (Molecular Devices, Sunnyvale, California, USA) amplifier and 1 X LU head stage were used. The preparations were bathed in saline for 250 sec during the stimulation paradigm; then, the bath was exchanged to one containing selenium selenite (5 mM) and stimulated for another 250 sec and finally, the bath was flushed with fresh saline once more and stimulated for another 250 sec. The amplitude of the EJPs was used for assessment[47,48].

Ethical consideration: Invertebrate animal care was approved by the Institutional Animal Care and Use Committee.

Statistical methods: In general, when normality was to be assumed, Shapiro-Wilk or Kruskal-Wallis ANOVA tests were used to validate the assumptions. The use of a t-test, a paired t-test or a Wilcoxon Signed-Rank Test was also used to determine significance.

For the survival analysis in *D. melanogaster*, Weibull regression was used to model the survival curves with the factors being solution type (Control, selenium selenite). For the behavioral studies, a t-test was used to determine the difference between the treatments (control, selenium selenite).

For the heart rate, crayfish MRO, crab PD and synaptic transmission at the NMJs of the crayfish and *Drosophila* experiments, the data consisted of an initial saline treatment followed by the application of selenium selenite treatment. Behaviors before and during exposure were compared by utilizing a paired t-test and in some cases, a Wilcoxon Signed Rank test if the data were not normally distributed. A significant level of 0.05 was used in all studies. The statistical analysis used is presented in the results for each procedure.

RESULTS

Survival and developmental studies of *Drosophila melanogaster*: When first-instar larvae were placed in standard cornmeal food tainted with 1 mM sodium selenite, they died within 24 hrs. Male adults 2 to 3 days post pupation that were placed in tainted vials died more rapidly than control larvae fed the same food mixed with water (Fig. 4; ANOVA, $p<0.0001$). Adult *Drosophila* fed tainted food survived approximately 3 days, with 50% of them surviving 2 and 1/2 days. The adults became lethargic and then immobile.

Larval behavioral assays-body wall movements and mouth hook movements: The 2nd instars, placed in standard cornmeal food and food containing 0.1 or 0.5 mM of sodium selenite, were assessed for behaviors after 24 hrs. Those exposed to 0.5 mM sodium selenite showed significant differences in both body wall movements and mouth hook movements (Fig. 5; $p<0.05$ ANOVA by Kruskal-Wallis followed Wilcoxon Signed-Rank test). The body wall movements significantly decreased for the 0.5 mM treatment (Mean±SEM; control 52±3; 0.1 mM 47±2; 0.5 mM 17±5). Likewise, mouth hook movements showed a similar trend with a significant effect for 0.5 mM treatment (Mean±SEM; control 109±7; 0.1 mM 126±3; 0.5 mM 45±13).

Beating rate of heart in larval *Drosophila*: The acute exposure of *in situ* larval hearts to sodium selenite at 1 mM or 5 mM did not show consistent changes in heart rate (Fig. 6a-b). Instead, some preparations showed a rate increase, while others exhibited a decrease. Upon flushing the preparation with fresh saline (not containing selenium selenite), the heart rates largely returned to approximately their initial levels. To normalize differences in the initial rates, a percent change was determined for each preparation and then the (Mean±SEM) of the percent change was graphed (Fig. 6c; 1 mM 15±16; 5 mM -10±7). There were no significant differences for either concentration ($p>0.05$, $n = 10$ for each concentration (Fig. 6c).

Crayfish cardiac function, survival and sensory responsiveness: Change in the crayfish heart rate can be used as an index for whether the animal is responsive to environmental stimuli. A physical touch on the telson or a disturbance in the water will trigger a brief pause, followed by an increase in beating rate (Fig. 2). Because the beating of the crayfish heart is neurogenic, damage to the CNS, sensory circuitry or modulating neurons (which communicate directly with the heart) will alter the effect of sensory stimulation; likewise, the overall rate, even without a sensory stimulus, can be altered by actions within the nervous system. Heart rate increased in all 6 control (i.e., injections containing saline only). One of the 6 experimental crayfish (i.e., injections containing selenium selenite) decreased, while all five others increased (Fig. 7a). After 20 min, all 6 in each group responded to a tap on the telson, either by exhibiting a tail-flip response or walking away from the stimulus; the controls also consistently showed an increase in the rate of beating, while not all the experimental crayfish did. After 4 hrs, all 6 of the experimental crayfish had died, while all 6 of the

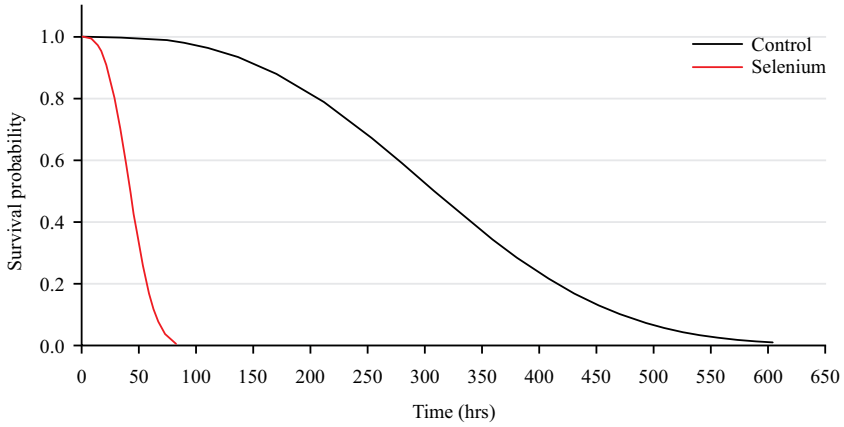

Fig. 4: Survival of adult *Drosophila* exposed to dietary sodium selenite

Ten vials, with ten adults (2-3 days post eclosion from pupa) in each vial, were tested with either normal food (control) or with food tainted with sodium selenite (1 mM). A significant difference was observed between the survival curves for the two conditions (ANOVA, $p<0.0001$). The selenite group died out at a quicker rate than the controls

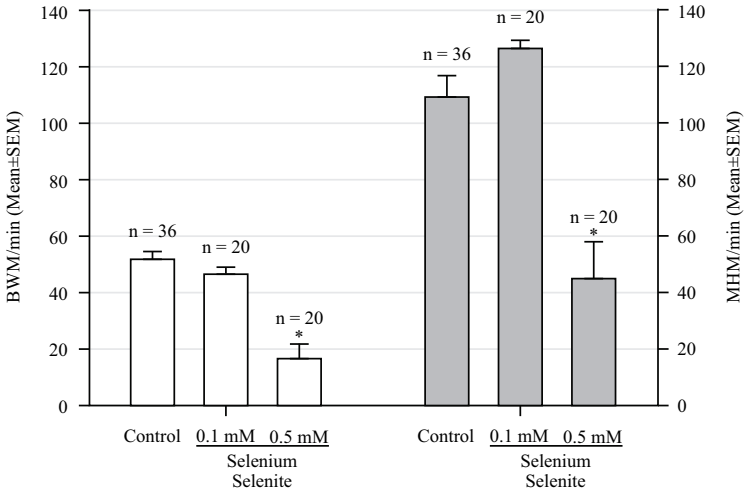

Fig. 5: Behavioral assays of larval body wall movements (BWM) and mouth hook movements (MHM) per min
These behaviors were significantly reduced after 24 hrs of eating food containing selenium selenite at 0.5 mM (*p<0.05, ANOVA, Wilcoxon signed-rank test), this effect was not observed at 0.1 mM

saline-injected controls survived; those that survived lived for up to two weeks, at which time observations were halted. A percent change in the rates for the initial responses to injection or after 20 min did not show significant differences (Fig. 7b; p>0.05; t-test; Saline injected had a Mean±SEM percent change right after have injection of 26±9 and after 20 min of 6±2, while selenium selenite injected crayfish had a percent change initially of 53±47 and after 20 min of -2±3).

Crayfish muscle receptor organ: The abdominal proprioceptive neurons associated with the crayfish muscle receptor organ (MRO) were not acutely affected by 5 mM sodium selenite. Even after 10 min of incubation (including sensory stimulation to ensure the opening of both stretch-activated channels in the sensory endings and ionic channels along the sensory axons), the overall activity observed was not affected as observed in the average number of spikes before and during exposure to sodium selenite (Fig. 8a). There were no statistical differences with exposure to sodium selenite (Fig. 8b; p>0.05; paired t-test, n = 6). In normalizing the initial differences in the neural activity among preparations, from the stimulation of moving the joints, a percent difference in the initial mean number of spikes was compared to the responses in the subsequent testing times. (Fig. 8c; Mean±SEM of the percent change from initial saline to sodium selenite -16±15 and after 10 min 12±11 followed by removal and flushing of preparation with fresh saline -9±5). Again, no statistically significant differences occurred (p>0.05; t-test, n = 6).

Crab chordotonal organ: The sensory activity of the proprioceptive PD organ in the crab leg increased upon exposure to and incubation of sodium selenite (5 mM) (Fig. 9a; p<0.05, paired t-test, n = 6). The stimulation paradigm used here was the same as was employed for the crayfish MRO (explained above). To normalize the activity among preparations, a percent change from the initial activity was used for analysis (Fig. 9b; Mean±SEM of the percent change from initial saline to sodium selenite 8±5 and after 10 min 17±6 followed by removal and flushing of preparation with fresh saline -3±7). Again, no statistically significant differences occurred (p>0.05; t-test, n = 6). There were no significant differences in percent change between the initial exposure to sodium selenite and after 10 min of incubation. The only differences observed were in the activity within preparations.

Synaptic transmission at crayfish and larval *Drosophila* neuromuscular junctions: The synaptic transmission observed at the NMJs of crayfish and larval *Drosophila* is similar in many ways. Both are very sensitive to alterations in the bathing medium's Ca^{2+} concentrations. This indicates that Ca^{2+} entry to the presynaptic nerve terminal initiates evoked vesicular fusion events. Additionally, both NMJs are glutamatergic with ionotropic postsynaptic quisqualate receptor subtypes. They produce graded excitatory postsynaptic junction potentials (EJPs)[47,48]. Increases in the concentration of free Mg^{2+} or Cd^{2+} in the bathing medium depress the amplitude of the EJPs in both preparations; thus, it was of interest to know whether sodium selenite might also have an effect. The ionic forms of free selenium (oxidation

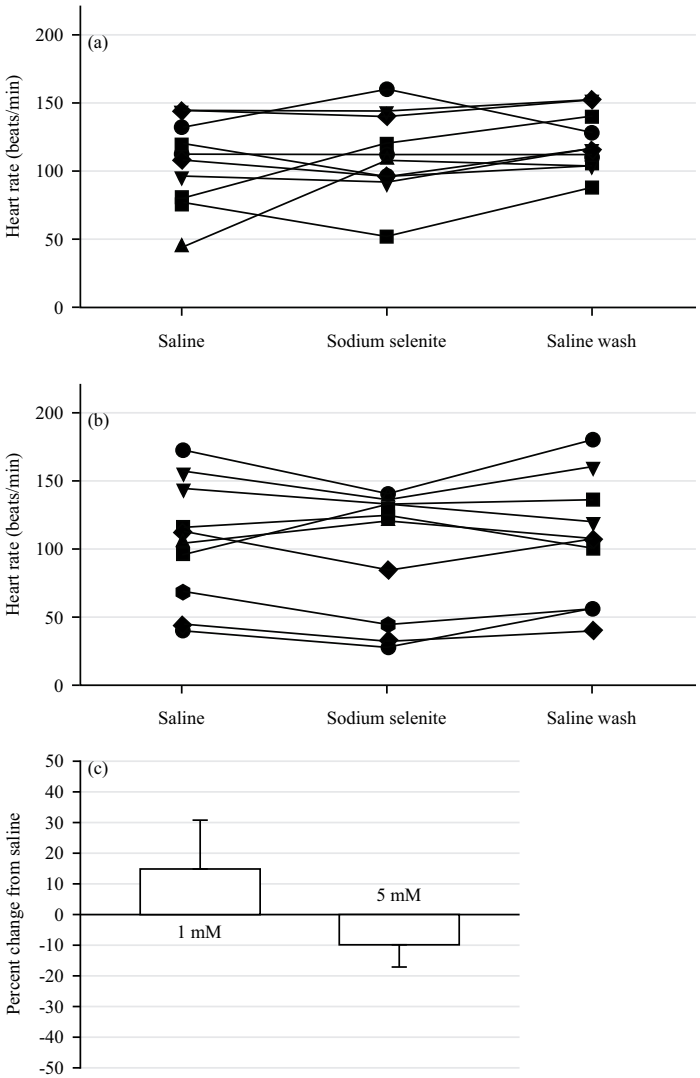

Fig. 6(a-b): Effects of acute exposure to sodium selenite on the *in situ* third-instar larval hearts, (a) Heart rate before, during and after exposure to 1 mM sodium selenite, (b) Heart rate before, during and after exposure to 5 mM sodium selenite and (c) Percent changes in the heart rates before and during exposure to sodium selenite

(a-b) No significant differences in heart rate were noted. The lines with symbols represent individual preparations in different conditions and (c) Rate per min was determined during exposure to sodium selenite and directly after a bath exchange to fresh saline. A paired t-test from initial saline to sodium selenite with a normality test (Shapiro-Wilk) revealed no significant differences for either concentration ($p>0.05$, n = 10 for each concentration)

states +2, +4 and +6) would not be expected to dissociate in the bathing medium. Synaptic transmission on the opener muscle of the crayfish preparation has relatively low output; it requires a facilitated response to obtain reliable measures of EJP amplitude, as it is necessary to examine how synaptic responses are affected by exposure to exogenous compounds (Fig. 10a).

Surprisingly, during exposure to selenium selenite (5 mM) over min, EJP amplitude at the crayfish NMJ slightly increased. A representative response is shown in Fig. 10a and the amplitude of the last EJP induced by the stimulus train over time. The amplitudes of the last EJP in saline and then during exposure to selenium selenite followed after flushing away the selenium selenite with fresh saline. The colored box indicates the time of exposure to selenium selenite (Fig. 10b) The responses for individual preparation are shown in Fig. 10c. There was a significant increase in the response exposed to selenium selenite ($p<0.05$, paired t-test, n = 6). There was an average of 14% increase (Fig. 10d; Mean±SEM of the percent change from initial saline to sodium selenite 14±4 and after flushing the preparation with fresh saline 17±8). As for synaptic transmission at the larval NMJs which have greater synaptic efficacy as compared to the crayfish NMJ used in this study, selenium selenite (5 mM) was observed to

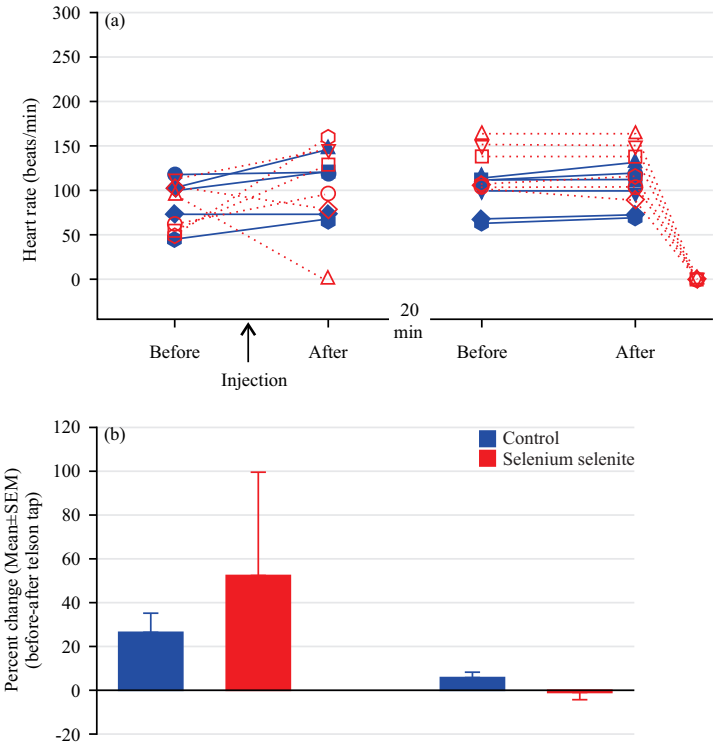

Fig. 7(a-b): Effects of sodium selenite injected into the hemolymph of crayfish, 6 crayfish were injected with saline and 6 crayfish were injected with sodium selenite, (a) Effect on heart rate (HR) with sensory stimuli before and right after injecting saline or sodium selenite and (b) Effect on heart rate (HR) with sensory stimuli after 20 min after injecting saline or sodium selenite

(a) All 12 crayfish responded to tail taps before and after injections, though one of the selenium selenite crayfish was lethargic immediately after injection, however, it was observed to be responsive after 20 min. After 4 hrs, all six of the selenium selenite-injected crayfish died. The crayfish injected with only saline survived for the full two weeks of observation and (b) A percent change was determined for each crayfish before and after a tap on the telson and Mean±SEM percent change is shown for each group of crayfish

depress the EJP amplitude. A representative response is shown in Fig. 11a. The amplitudes of the EJP in saline and then during exposure to selenium selenite followed after flushing away the selenium selenite with fresh saline. The colored box indicates the time of exposure to selenium selenite (Fig. 11b). The responses for individual preparation are shown in Fig. 11c. There was a significant increase in the response exposed to selenium selenite ($p<0.05$, paired t-test, $n = 9$). This resulted in an average decrease of 32 % (Fig. 11d; Mean±SEM of the percent change from initial saline to sodium selenite -32±11 and after flushing the preparation with fresh saline -14±4).

Reproducibility: To examine reproducibility, participants in an undergraduate course analyzed three given files of electrical activity from the crab PD organs. Similar trends were revealed without significant differences. The absolute counts in the number of spikes were slightly different due to choosing different thresholds in the analysis of the spikes from the baseline, but the same trends were observed (Fig. 12a-c). Data sets previously analyzed by a person who has conducted all the analysis of the crab PD preparation presented earlier in this study were compared the analysis to 3 different pairs of individual students in a neurophysiology course with brief training in the analysis procedures. A pair was provided with one data set. One pair analyzed Fig. 12a data set another Fig. 12b data set and the 3rd pair Fig. 12c data set. The well-trained individual set a threshold based on the overview of the entire data set so as not to potentially detect the noise of the baseline or signals close to the baseline. All three pairs had very similar trends in the analysis except the pair provided Fig. 12a data set with the data during the saline exposure. Upon reviewing the window discriminator threshold used for analysis it was determined this analysis of detecting more spikes was due to picking up the very small signals close to the baseline for that time period which did not appear

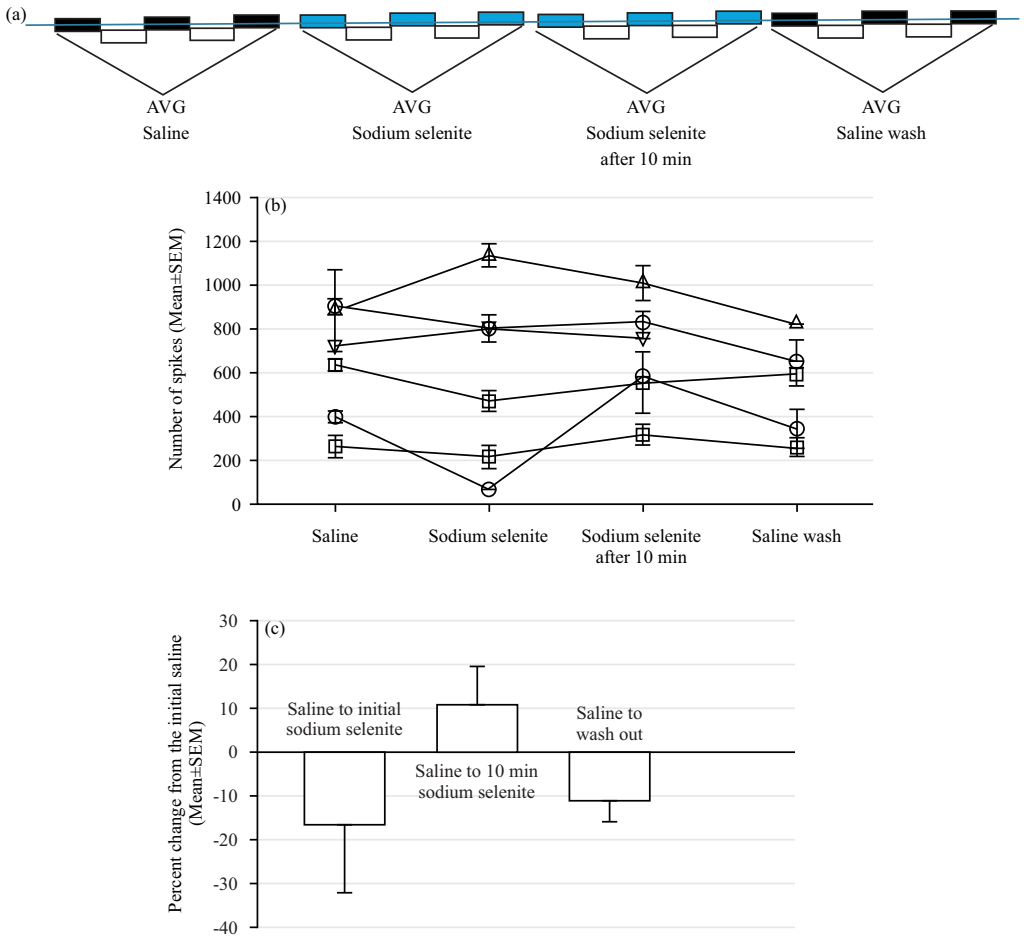

Fig. 8(a-c): Effect of sodium selenite on stimulated sensory nerve activity at the crayfish muscle receptor organ, (a) Schematic procedure for the movements of the abdominal segment and the exposure to the MRO, (b) Activity of the MRO nerve before and during exposure to sodium selenite and (c) Percent change in the neural activity with exposure to sodium selenite

(a) Closed boxes represent the number of spikes measured in 10 sec windows for three trials of each condition: Saline, sodium selenite (5 mM), sodium selenite (post-incubation of 10 min) and washout with fresh saline. The joints were returned to the initial position between each trial and during the 10 min incubation (open boxes). The neural activity was measured for each exposure and averaged. Each movement was separated by a minimum of 10 sec, which elapsed while the joint was held in a relaxed position, (b) Number of spikes in each of the three trials (±SEM). There was no significant change observed with acute exposure or after the 10 min exposure to sodium selenite (5 mM) as compared to the initial activity ($p>0.05$; paired t-test; $n = 6$). The lines with symbols represent individual preparations in different conditions and (c) Averaged activity from the initial values within each preparation also indicated no significant effect on sodium selenite (5 mM) exposure (Rank sum Sign test; $n = 6$).

during the rest of the experimental duration. Injection of sodium selenite (5 mM) into the hemolymph of a crayfish showed the same observations as previous recordings with a slight increase in HR with a sensory stimulus (tap on the telson), but after 4 hrs from the injection time, the animal died (Fig. 13).

DISCUSSION

Dietary consumption of sodium selenite in tainted food for larval and adult *Drosophila* would indicate that it is toxic at 1 mM, but it is not yet known how the toxicity is induced. Given that body wall movements and mouth hook movements were reduced in 24 hrs but not entirely inhibited, larvae and adults might not have been able to sustain their dietary needs for survival due to lack of food intake. It appears as though sodium selenite is absorbed across the intestinal tract to produce such behavioral responses and mortality; however, a measure of systemic levels of sodium selenite was not obtained. It is possible that the larvae and adults reduced their eating intake to avoid the tainted food. This theory may be addressed through future studies with dye in the tainted food to confirm content in larval and adult intestine.

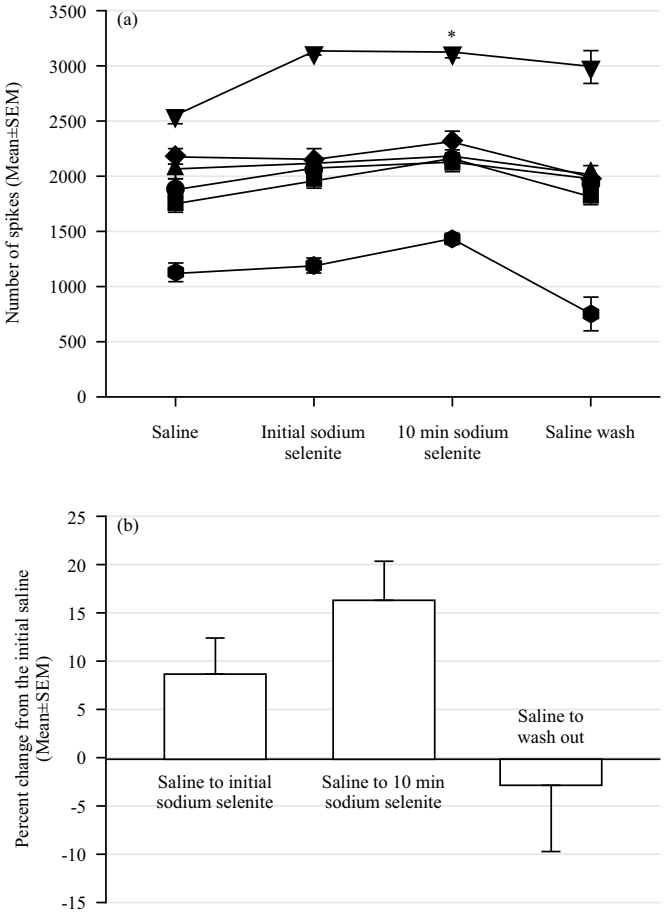

Fig. 9(a-b): Effect of sodium selenite on stimulated sensory nerve activity of the propodite-dactylopodite (PD) chordotonal organ in the walking leg of the crab, (a) Activity of the PD nerve before and during exposure to sodium selenite and (b) Percent change in the neural activity with exposure to sodium selenite

Joint was successively extended and flexed (10 sec each) thrice in each condition: Saline, sodium selenite (5 mM), sodium selenite (after 10 min incubation) and washout with fresh saline, (a) Neural activity was measured for three trials and averaged (±SEM). Significant increase in activity was observed for the acute exposure and after 10 min exposure to sodium selenite (5 mM) as compared to the initial activity ($p<0.05$; paired t-test; for the group of $n = 6$). The lines with symbols represent individual preparations in different conditions and (b) Percent change in the averaged activity from the initial values within each preparation also indicated that exposure to sodium selenite (5 mM) led to a significant effect ($p<0.05$; Rank sum Sign test; $n = 6$)

Given that the crayfish were directly injected with sodium selenite, there is no doubt that the circulating levels in the hemolymph were a direct cause of the relatively rapid death. Control crayfish were injected with the same saline as that used for the sodium selenite injections and these six crayfish survived two weeks of monitoring; this confirms that neither the stress caused by the injections nor the placement of the leads in the heart caused the rapid death observed in the crayfish injected with sodium selenite. The mechanism behind the crayfish deaths is not yet established. Within the 20 min following injections, all crayfish were responsive to a telson tap, indicating that sensory neurons were still functional, as well as the CNS, motor neurons, skeletal muscles and synaptic transmission to the neurogenic heart. At 4 hrs post-injection, the examination of crayfish injected with sodium selenite saw no movement, no response to sensory stimulation and no heartbeat measured via the impedance technique.

Future studies with longer exposure times and recordings taken at the NMJs to examine sensory functions and synaptic transmission would help resolve whether these physiological functions were compromised. This could be obtained via *in situ* studies with monitoring taking place over hours, days, or right after injections into the hemolymph; however, the safety protocols provided by this investigation's selenium supplier (Sigma-Aldrich) advised heightened caution regarding sodium selenite vapors due to their toxicity. There

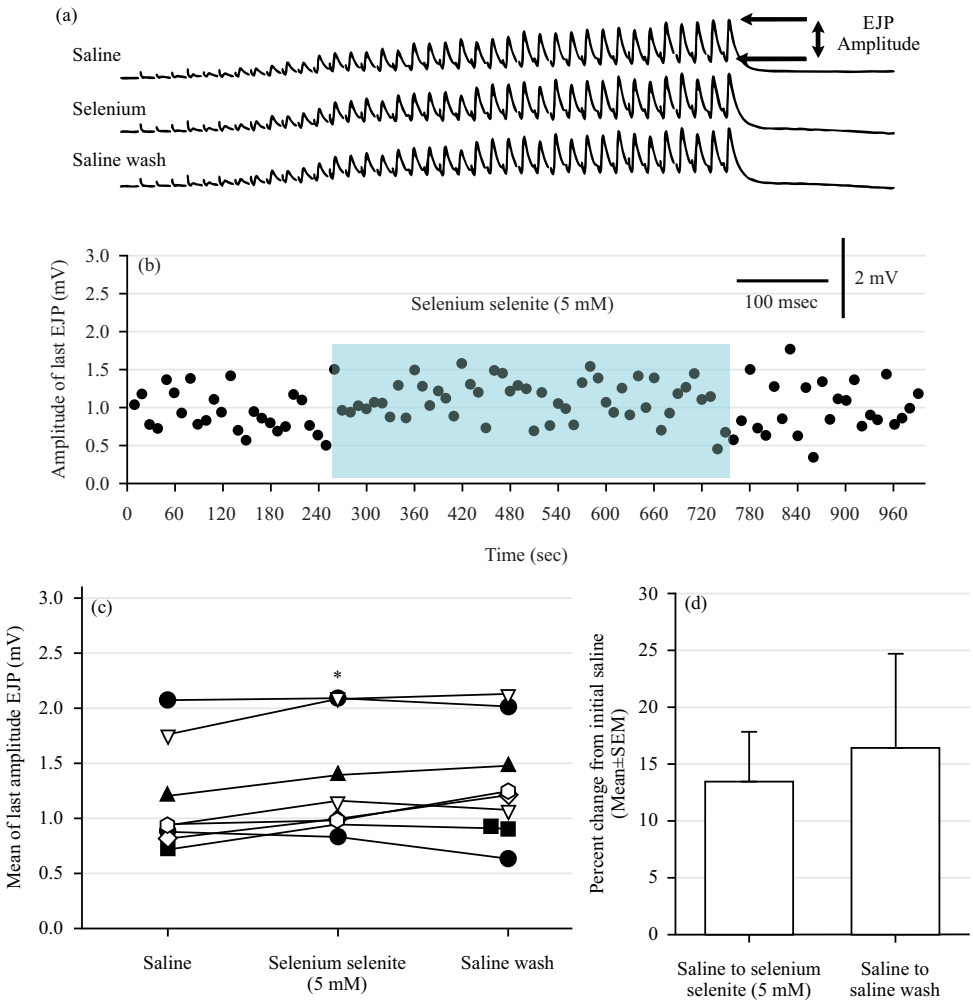

Fig. 10(a-d): Effects of sodium selenite on evoked excitatory junction potentials (EJPs) at the neuromuscular junction of the opener muscle in the walking leg of crayfish, (a) EJPs before and after exposure to sodium selenite, (b) Representative preparation of the amplitudes of the EJPs used for analysis, (c) Average in the EJPs amplitudes for each preparation used for analysis and (d) Percent difference in the amplitudes of EJPs when exposed and when sodium selenite was flushed away

(a) Motor nerve was stimulated every 10 sec with pulses of 60 Hz trains in saline, while bathed in sodium selenite (5 mM) and during washout in fresh saline, (b) Throughout the paradigm, the last EJP amplitude within the train was measured, (c) Average amplitude of the last EJP during the 250 trials in saline, the last 250, 500 trials with sodium selenite exposure and the 250 trials in the saline wash were used for comparative measures. The amplitudes of the EJPs were significantly increased during exposure to sodium selenite (paired t-test from initial saline to selenium selenite with normality test by Shapiro-Wilk; $p<0.05$; $n = 6$). The lines with symbols represent individual preparations in different conditions and (d) Normalizing the variation in initial values by determining a mean percent change from saline to sodium selenite for each preparation illustrated a small average change of 14% and increased amplitude was generally maintained after the preparation was rinsed with fresh saline

are no reports on how sodium selenite responds to the physiological salines used in this study or if vapors are produced from that combination, so we minimized exposure to the preparations as much as possible during experimentation. Likewise, no information exists on the action of sodium selenite in the hemolymph of the animals used and it is yet unknown whether the compound binds to proteins and tissues in the animals, so the systemic circulating concentration assumed for the injections might be lower than anticipated. In both saline and hemolymph, the sodium selenite may remain in salt form (i.e., Na_2SeO_3); though it is noted that the pentahydrate form (Na_2SeO_3 $(H_2O)_5$) is the most common water-soluble form[49]. The selenium may thus be low in osmolarity in the crayfish and *Drosophila* saline as compared to the high osmolarity of the salts in crab saline. Sodium selenite likely does not appear in solution as any of

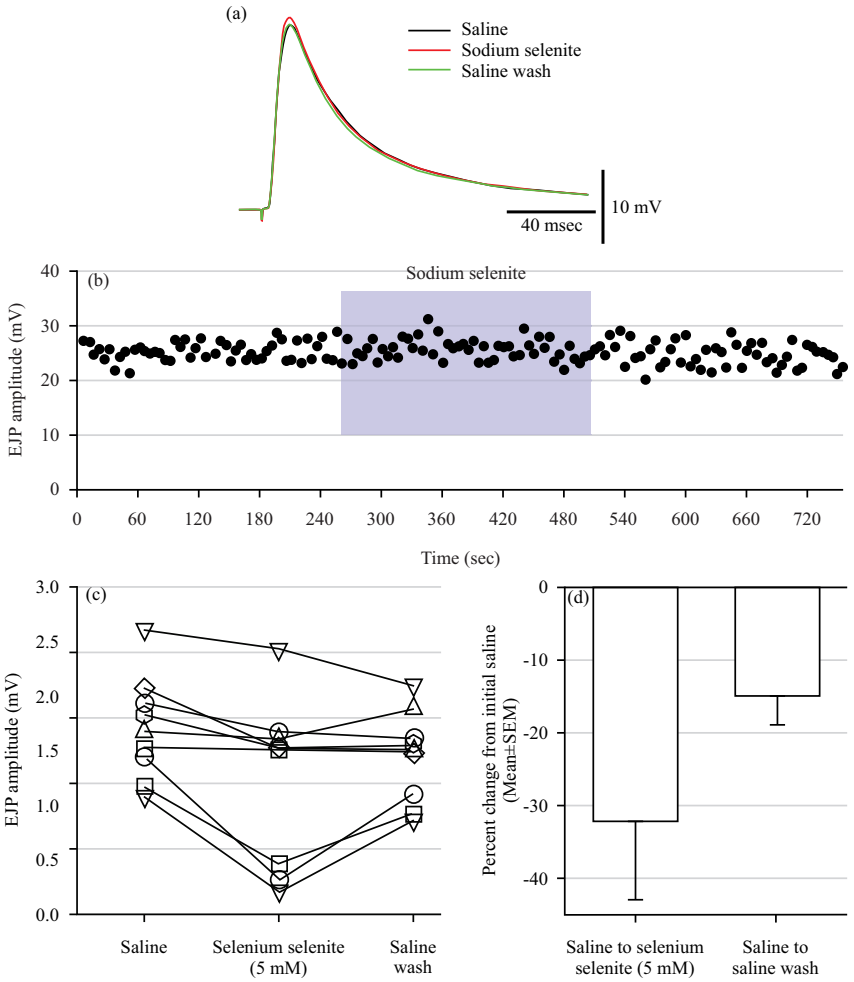

Fig. 11(a-d): Effects of selenium selenite (5 mM) on evoked excitatory junction potentials (EJPs) at the larval *Drosophila* neuromuscular junction, (a) EJPs before and after exposure to sodium selenite, (b) A representative preparation of the amplitudes of the EJPs used for analysis, (c) Average in the EJPs amplitudes for each preparation and (d) Percent difference in the amplitudes of EJPs when exposed and when sodium selenite was flushed away

(b) Segmental nerve was stimulated at a rate of 0.5 Hz in saline, during exposure to selenium selenite and after a rinse with fresh saline, (c) Average amplitude of the EJPs for each of the nine preparations is shown for each condition. The lines with symbols represent individual preparations in different conditions and (d) In normalizing the variation in the initial values by determining a mean percent change from saline to selenium selenite for each preparation, an average change of 32% was observed. Generally, preparations regained some EJP amplitude after the rinsing of the preparation with fresh saline

its free ions: selenium (oxidation state 0), selenide (-2), selenite (+4) and selenate (+6), which suggests that it is unlikely for sodium selenite to directly affect ion channels as Fe^{3+}, Cd^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+} or Zn^{2+} affect channels for Na^{+}, K^{+} or Ca^{2+} ion flux[58].

The acute enhancement of synaptic transmission at the crayfish NMJ is not likely due to activation of second messenger systems, such as IP3/ DAG (known to be affected by serotonin[50]; as the heightened response returns to baseline after removal of sodium selenite in most preparations. Thus, contrary to what was stated above, sodium selenite may promote presynaptic Ca^{2+} entry and increase e voked vesicular fusion events. It may also be acting post-synaptically to directly enhance the response of the glutamate receptors or acting on ion channels in the muscle fibers to produce larger EJPs. Future studies examining the amplitudes of single spontaneous quantal events, how frequently they occur and the input resistance of the muscle fiber would clarify the mechanism of action on this preparation. The decrease in synaptic transmission at the larval *Drosophila* NMJ could be due to either pre- or post-synaptic actions; likewise, studies similar to those needed to further examine the crayfish NMJ would be required to address the mechanism of action at that of larval *Drosophila*.

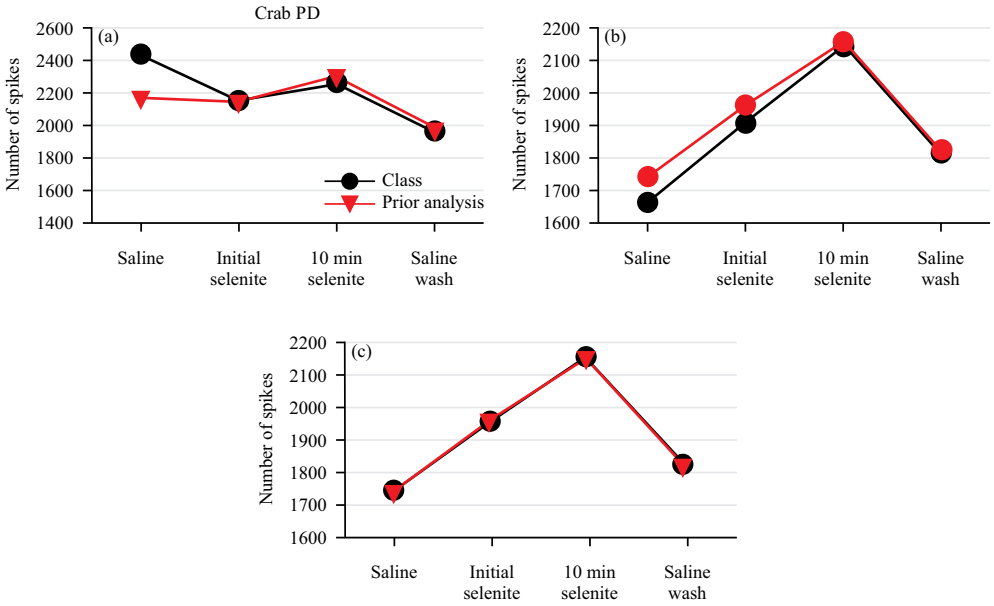

Fig. 12(a-c): (a-c) Three different data sets of electrical activity from three different preparations of the crab PD organ were reanalyzed to compare reproducibility in analysis. A single preparation analyzed by a well-trained individual and repeated by novice students within a class. Trends in the analysis are similar except for the initial data point in saline due to choosing a higher sensitivity of threshold discriminators for counting the spike in neural activity

A detailed analysis by one who has been conducting the earlier analysis (red) and analysis made by students in a course (black). The variation in the absolute values is due to choosing different thresholds from the baseline in counting the smaller amplitude spikes and lines with symbols represent individual preparations in different conditions

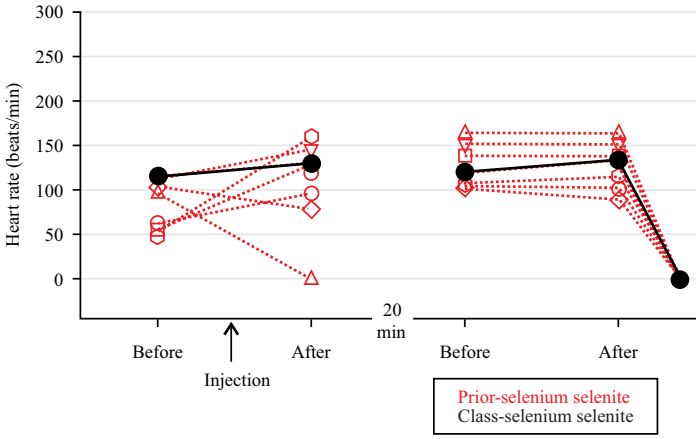

Fig. 13: Injection of sodium selenite (5 mM) into the hemolymph of a crayfish

Results were the same observations as previous recordings with a sensory stimulus (tap on the telson) before and after injection, as well as after 4 hrs from the injection time the animal died. The lines with symbols represent individual preparations in different conditions

The primary sensory neurons of the crayfish MRO and crab PD organ provide direct insight into the action on neurons without the additional confounding complexity of synaptic processes. The sensory neurons are activated by mechanical transduction at the sensory endings through stretch-activated channels, resulting in depolarization of the neurons and producing spikes that can be measured. The crayfish MRO did not produce consistent responses. Some preparations saw increased spike production in the presence of sodium selenite, while others saw a decrease. Generally, the neural activity of the crab PD organ increased when exposed to sodium selenite. Sodium selenite could directly affect stretch-activated ion channels that, in turn, alter the biophysical properties of the involved sensory neurons, their

effects on membrane potential, and their threshold of activation. Future studies could be performed by removing the sensory ending from the crab preparation and examining whether changes in the CAP activation threshold occur in the presence of sodium selenite.

The acute illnesses and toxicity associated with selenium poisoning (i.e., selenosis) in humans may occur due to similar mechanisms to those presented herein, albeit over a longer period of exposure. Death upon excessive, acute ingestion of supplemental 'therapeutic' selenium powder occurred via cardiac arrest[51], which may be due to the element's direct effects on electrical pacing cells or overall cell activity for contraction maintenance. The wide range of symptoms observed in humans is likely due to an accumulation of effects that affect many cells in many different ways; after all, individual cell types feature considerably different distributions of surface proteins and ion channel types that could be potential targets for direct action on cells. The levels of selenium recommended for long periods of use may also show some pathological consequences if excretion or abnormal accumulation in tissues occurs.

In mammals, selenium present as Selenoprotein P (Sepp1) is highly regulated by the liver but is taken up at higher levels into the brain and testis due to transport by Apolipoprotein E Receptor-2 (apoER2)[52]. Transport across and into cells of other animals is still an area of open investigation[53]. Interesting pollination ecology has demonstrated that plants with higher levels of selenium may grow better, but this can also reduce the pollination success of plants as insects may be deterred from approaching. Dietary consumption of various concentrations of selenium in *Drosophila* genetically modified to feature chemosensory inhibition of food would be an interesting future study to address whether taste is a key factor in this avoidance. It is also possible that different forms of inorganic and organic selenium-containing compounds may illustrate altered plant-insect attraction or effects on animal physiology or behavior. This study may indeed have raised more questions than it has answered, but it has nonetheless raised some interesting findings and has emphasized the importance of comparative, inter-species approaches in physiological research.

In examining reproducibility of this investigation, a group of students in a neurophysiology class conducted some of the same experiments and data analysis of their own data as well as replicated data analysis blinded to the experimental design of previous recorded experiments. Joint displacement rate during recordings from the crayfish MRO or crab leg PD nerve may have varied amongst the groups. Data sets were separated from previous recordings in the research laboratory. Class participants replicated conditions of the crab PD nerve recording and the crayfish MRO, as well as BWM and MHM for larval *Drosophila*. This is an approach to independently review results by experimentation by other investigators before publication and to provide students with ACURE (authentic course-based undergraduate research experiences)[54-56], which is an approach that builds on the CURE (course-based undergraduate research experiences) philosophy[57]. Analysis of spike occurrence in the crab PD organ was similar among different participants. However, the variability in analysis suggests that data sets provided for the study may require samples with illustrations and details on analysis. Especially when thresholds of signals from background activity are used.

CONCLUSION

The overall results of this investigation into the acute effects of sodium selenite on larval *Drosophila* physiological function, survival and development as well as physiological processes in crabs and crayfish indicate that there are differential effects on the tissues of various invertebrate species. Generally, consumption of food tainted with or systemic exposure to 1 mM sodium selenite is toxic to both *Drosophila* larvae and adults within a day and lethal to crayfish within a few hours. Surprisingly, acute exposure to the crab PD organ increased activity. A mixture of neural responses (increasing vs. decreasing) was observed at the crayfish MRO. Even more surprising was the acute increase in synaptic responses observed at the crayfish NMJ, particularly when accompanied by reduced synaptic transmission at the larval *Drosophila* NMJ.

SIGNIFICANCE STATEMENT

Since there are various means of selenium exposure, it is interesting to investigate how overexposure affects animals. This study provided a comprehensive survey of how a few model organisms respond in conditions of excess selenium exposure to allow for further detailed research on cellular mechanisms. In crayfish, crab and *Drosophila* sensory function can be and impacted as well as survival. This is a significant study for preliminarily understanding the physiological effects of selenium on model organisms so that selenium's actions on other organisms may be assessed.

ACKNOWLEDGMENT

Use of classroom teaching equipment by the Department of Biology, University of Kentucky. Chellgren Endowed Funding (R.L.C.). United States Military Academy for publication cost.

REFERENCES

1. Okunieff, P., S. Swarts, P. Keng, W. Sun and W. Wang *et al*., 2008. Antioxidants Reduce Consequences of Radiation Exposure. In: Oxygen Transport to Tissue XXIX, Kang, K.A., D.K. Harrison and D.F. Bruley (Eds.), Springer, Boston, Massachusetts, ISBN: 978-0-387-74911-2, pp: 165-178.
2. Zoidis, E., I. Seremelis, N. Kontopoulos and G.P. Danezis, 2018. Selenium-dependent antioxidant enzymes: Actions and properties of selenoproteins. Antioxidants, Vol. 7. 10.3390/antiox7050066.
3. Gorini, F., L. Sabatino, A. Pingitore and C. Vassalle, 2021. Selenium: An element of life essential for thyroid function. Molecules, Vol. 26. 10.3390/molecules26237084.
4. Jiang, K., C. Xie, Z. Li, H. Zeng, Y. Zhao and Z. Shi, 2022. Selenium intake and its interaction with iron intake are associated with cognitive functions in Chinese adults: A longitudinal study. Nutrients, Vol. 14. 10.3390/nu14153005.
5. Chen, Z., Y. Lu, X. Dun, X. Wang and H. Wang, 2023. Research progress of selenium-enriched foods. Nutrients, Vol. 15. 10.3390/nu15194189.
6. Guo, Q., J. Ye, J. Zeng, L. Chen, H. Korpelainen and C. Li, 2023. Selenium species transforming along soil-plant continuum and their beneficial roles for horticultural crops. Hortic. Res., Vol. 10. 10.1093/hr/uhac270.
7. Ahmad, M.A., M. Ali, S. Saeed and F. Nawaz, 2023. Effect of selenium accumulation on foraging behavior of pollinators and seed yield in *Trifolium alexandrinum* L. Environ. Sci. Pollut. Res., 30: 33438-33445.
8. Cummins, L.M. and E.T. Kimura, 1971. Safety evaluation of selenium sulfide antidandruff shampoos. Toxicol. Appl. Pharmacol., 20: 89-96.
9. Gebreeyessus, G.D. and F. Zewge, 2019. A review on environmental selenium issues. SN Appl. Sci., Vol. 1. 10.1007/s42452-018-0032-9.
10. Guan, L., B. Han, Z. Li, F. Hua and F. Huang *et al*., 2009. Sodium selenite induces apoptosis by ROS-mediated endoplasmic reticulum stress and mitochondrial dysfunction in human acute promyelocytic leukemia NB4 cells. Apoptosis, 14: 218-225.
11. Stroikova, V., D. Regul and B. Meder, 2021. Rare case of selenite poisoning manifesting as non-ST-segment elevation myocardial infarction. JACC: Case Rep., 3: 811-815.
12. Alexander, J., 2015. Selenium. In: Handbook on the Toxicology of Metals, Nordberg, G.F., B.A. Fowler and M. Nordberg (Eds.), Academic Press, Cambridge, Massachusetts, ISBN: 9780444594532, pp: 1175-1208.
13. Maier, K.J. and A.W. Knight, 1994. Ecotoxicology of Selenium in Freshwater Systems. In: Reviews of Environmental Contamination and Toxicology, Ware, G.W. (Ed.), Springer, New York, ISBN: 978-1-4684-7068-0, pp: 31-48.
14. Ogle, R.S. and A. W. Knight, 1996. Selenium bioaccumulation in aquatic ecosystems: 1. Effects of sulfate on the uptake and toxicity of selenate in *Daphnia magna*. Arch. Environ. Contam. Toxicol., 30: 274-279.
15. Chapman, P.M., 1999. Invited debate/commentary: Selenium-a potential time bomb or just another contaminant? Hum. Ecol. Risk Assess.: Int. J., 5: 1123-1138.
16. Jarvinen, A.W. and G.T. Ankley, 1999. Linkage of Effects to Tissue Residues: Development of a Comprehensive Database for A quatic Organisms Exposed to Inorganic and Organic Chemicals. Society of Environmental Toxicology and Chemistry, Washington, DC, United States, ISBN: 9781880611135, Pages: 358.
17. Waqas, W., Y. Yuan, S. Ali, M. Zhang and M. Shafiq *et al*., 2024. Toxic effects of heavy metals on crustaceans and associated health risks in humans: A review. Environ. Chem. Lett., 22: 1391-1411.
18. Graves, S.D., K. Liber, V. Palace, M. Hecker and D.M. Janz, 2022. Response of crustacean zooplankton and benthic macroinvertebrate communities to selenium additions in a boreal lake. Environ. Toxicol. Chem., 41: 95-107.
19. Listerman, L.R., J. Deskins, H. Bradacs and R.L. Cooper, 2000. Heart rate within male crayfish: Social interactions and effects of 5-HT. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol., 125: 251-263.
20. Schapker, H., T. Breithaupt, Z. Shuranova, Y. Burmistrov and R.L. Cooper, 2002. Heart and ventilatory correlative measures in crayfish during environmental disturbances and social interactions. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol., 131: 397-407.
21. Shuranova, Z.P., Y.M. Burmistrov, J.R. Strawn and R.L. Cooper, 2006. Evidence for an autonomic nervous system in decapod crustaceans. Int. J. Zool. Res., 2: 242-283.
22. Jan, L.Y. and Y.N. Jan, 1976. Properties of the larval neuromuscular junction in *Drosophila melanogaster*. J. Physiol., 262: 189-214.
23. Kurdyak, P., H.L. Atwood, B.A. Stewart and C.F. Wu, 1994. Differential physiology and morphology of motor axons to ventral longitudinal muscles in larval *Drosophila*. J. Comp. Neurol., 350: 463-472.
24. de Castro, C., J. Titlow, Z.R. Majeed and R.L. Cooper, 2014. Analysis of various physiological salines for heart rate, CNS function, and synaptic transmission at neuromuscular junctions in *Drosophila melanogaster* larvae. J. Comp. Physiol. A, 200: 83-92.

25. Johnstone, A.F.M. and R.L. Cooper, 2006. Direct innervation of the *Drosophila melanogaster* larval aorta. Brain Res., 1083: 159-163.
26. Elliott, E.R., K.E. Brock, A.C. Taul, A. Asadipooya and D. Bocook *et al*., 2023. The effects of zinc on proprioceptive sensory function and nerve conduction. NeuroSci, 4: 305-318.
27. Pankau, C., J. Nadolski, H. Tanner, C. Cryer and J.D. Girolamo *et al*., 2022. Examining the effect of manganese on physiological processes: Invertebrate models. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., Vol. 251. 10.1016/j.cbpc.2021.109209.
28. Wagers, M.L., A. Starks, J. Nadolski, S.M. Bierbower, S. Altenburg, B. Schryer and R.L. Cooper, 2024. Examining the effect of iron (ferric) on physiological processes: Invertebrate models. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., Vol. 278. 10.1016/j.cbpc.2024.109856.
29. Yamaguchi, M. and H. Yoshida, 2018. *Drosophila* as a Model Organism. In: Drosophila Models for Human Diseases, Yamaguchi, M. (Ed.), Springer, Singapore, ISBN: 978-981-13-0529-0, pp: 1-10.
30. So, J., D.H. Choe, M.K. Rust, J.T. Trumble and C.Y. Lee, 2023. The impact of selenium on insects. J. Econ. Entomol., 116: 1041-1062.
31. Ugur, B., K. Chen and H.J. Bellen, 2016. *Drosophila* tools and assays for the study of human diseases. Dis. Models. Mech., 9: 235-244.
32. Mackay, T.F.C. and R.R.H. Anholt, 2006. Of flies and man: *Drosophila* as a model for human complex traits. Annu. Rev. Genomics Hum. Genet., 7: 339-367.
33. Bier, E. and R. Bodmer, 2004. *Drosophila*, an emerging model for cardiac disease. Gene, 342: 1-11.
34. Wolf, M.J., H. Amrein, J.A. Izatt, M.A. Choma, M.C. Reedy and H.A. Rockman, 2006. *Drosophila* as a model for the identification of genes causing adult human heart disease. Proc. Natl. Acad. Sci. U.S.A., 103: 1394-1399.
35. Souidi, A. and K. Jagla, 2021. *Drosophila* heart as a model for cardiac development and diseases. Cells, Vol. 10. 10.3390/cells10113078.
36. Wagers, M.L., A. Starks, M.O. Abul-Khoudoud, S.M. Ahmed and A.W. Alhamdani *et al*., 2023. An invertebrate model in examining the effect of acute ferric iron exposure on proprioceptive neurons. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., Vol. 266. 10.1016/j.cbpc.2023.109558.
37. Elliott, E.R., J. Nadolski, R.D. McIntosh, M. Datta and D. Crawford *et al*., 2023. Investigation regarding the physiological effects of zinc on *Drosophila* and crawfish cardiac, neural, synaptic and behavioral processes. J. Pharmacol. Toxicol., 18: 166-189.
38. Cooper, A.S., K.E. Rymond, M.A. Ward, E.L. Bocook and R.L. Cooper, 2009. Monitoring heart function in larval *Drosophila melanogaster* for physiological studies. J. Visualized Exp., Vol. 33. 10.3791/1596.
39. Stewart, B.A., H.L. Atwood, J.J. Renger, J. Wang and C.F. Wu, 1994. Improved stability of *Drosophila* larval neuromuscular preparations in haemolymph-like physiological solutions. J. Comp. Physiol. A., 175: 179-191.
40. Bierbower, S.M. and R.L. Cooper, 2009. Measures of heart and ventilatory rates in freely moving crayfish. J. Visualized Exp., Vol. 32. 10.3791/1594.
41. Gleeson, R.A. and P.L. Zubkoff, 1977. The determination of hemolymph volume in the blue crab, *Callinectes sapidus*, utilizing ^{14}C-thiocyanate. Comp. Biochem. Physiol. Part A: Physiol., 56: 411-413.
42. Guirguis, M.S. and J.L. Wilkens, 1995. The role of the cardioregulatory nerves in mediating heart rate responses to locomotion, reduced stroke volume and neurohormones in *Homarus americanus*. Biol. Bull., 188: 179-185.
43. Leksrisawat, B., A.S. Cooper, A.B. Gilberts and R.L. Cooper, 2010. Muscle receptor organs in the crayfish abdomen: A student laboratory exercise in proprioception. J. Visualized Exp., Vol. 2010. 10.3791/2323.
44. Baierlein, B., A.L. Thurow, H.L. Atwood and R.L. Cooper, 2011. Membrane potentials, synaptic responses, neuronal circuitry, neuromodulation and muscle histology using the crayfish: Student laboratory exercises. J. Visualized Exp., Vol. 47. 10.3791/2322.
45. Majeed, Z.R., J. Titlow, H.B. Hartman and R. Cooper, 2013. Proprioception and tension receptors in crab limbs: Student laboratory exercises. J. Visualized Exp., Vol. 80. 10.3791/51050.
46. Cooper, A.S. and R.L. Cooper, 2009. Historical view and physiology demonstration at the NMJ of the crayfish opener muscle. J. Visualized Exp., Vol. 33. 10.3791/1595.
47. Lee, J.Y., D. Bhatt, D. Bhatt, W.Y. Chung and R.L. Cooper, 2009. Furthering pharmacological and physiological assessment of the glutamatergic receptors at the *Drosophila* neuromuscular junction. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., 150: 546-557.
48. Titlow, J.S. and R.L. Cooper, 2018. Glutamatergic Synthesis, Recycling, and Receptor Pharmacology at *Drosophila* and Crustacean Neuromuscular Junctions. In: Biochemical Approaches for Glutamatergic Neurotransmission, Parrot, S. and L. Denoroy (Eds.). Humana Press, New York, ISBN: 978-1-4939-7227-2, pp: 263-291.
49. Langner, B.E., 2000. Selenium and Selenium Compounds. In: Ullmann's Encyclopedia of Industrial Chemistry, Ullmann, F. (Ed.), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, ISBN: 9783527306732.
50. Wu, W.H. and R.L. Cooper, 2012. Serotonin and synaptic transmission at invertebrate neuromuscular junctions. Exp. Neurobiol., 21: 101-112.
51. See, K.A., P.S. Lavercombe, J. Dillon and R. Ginsberg, 2006. Accidental death from acute selenium poisoning. Med. J. Aust, 185: 388-389.

52. Olson, G.E., V.P. Winfrey, S.K. NagDas, K.E. Hill and R.F. Burk, 2007. Apolipoprotein E receptor-2 (ApoER2) mediates selenium uptake from selenoprotein P by the mouse testis. J. Biol. Chem., 282: 12290-12297.
53. Burk, R.F. and K.E. Hill, 2009. Selenoprotein P-expression, functions, and roles in mammals. Biochim. Biophys. Acta, Gen. Subj., 1790: 1441-1447.
54. Turkyilmaz-van der Velden, Y., N. Dintzner and M. Teperek, 2020. Reproducibility starts from you today. Patterns, Vol. 1. 10.1016/j.patter.2020.100099.
55. Malloy, C., V. Dayaram, S. Martha, B. Alvarez and I. Chukwudolue *et al*., 2017. The effects of potassium and muscle homogenate on proprioceptive responses in crayfish and crab. J. Exp. Zool. A: Ecol. Integr. Physiol., 327: 366-379.
56. Gard, J.E., K.E. Brock, E.R. Elliott, A.C. Taul, J. Nadolski et al., 2025. Investigation regarding the physiological effects of cobalt on physiological functions in Drosophila, crayfish, and crab: Behavioral, cardiac, neural, and synaptic properties. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., 10.1016/j.cbpc.2025.110165.
57. Linn, M.C., E. Palmer, A. Baranger, E. Gerard and E. Stone, 2015. Undergraduate research experiences: Impacts and opportunities. Science, Vol. 347. 10.1126/science.1261757.