CHAPTER 5

DISCUSSION

5.1 Physico-chemical parameters

The stream velocity at each elevation was not significantly different because all sites were located at plain areas, except at 900 mAMSL of both streams located behind small waterfalls. Then velocities at 900 mAMSL were higher eventhough there were not much water in stream in dry season. In rainy and cold seasons when water were increased, stream velocity at 700 mAMSL was not significant different with 900 mAMSL. Stream velocities in each season were not significantly different but there were tendencies to have the highest stream velocity in rainy season. There were trend of higher stream velocity in the control stream than that of Mae Sa Noi stream because measuring point of the control stream was shorter distance from waterfall than that of Mae Sa Noi. Hence, the control had higher slope and higher velocity than that of Mae Sa Noi. Consequently, velocities between streams was significantly different in mean comparison (Table 4.2) but this difference had not enough influence in cluster analysis to separate the grouping of the two streams since this method take all parameters into consideration.

Temperatures of water at each elevation were not significantly different because the water mixed well in flowing water. Temperatures were significantly changed following season, because of the influence of natural climatic change. Further more the 2 streams locate on the same mountain range and having similar climate.

The increasing of conductivities and TDS along both streams caused from the accumulation of contaminants along the distance of the streams. In rainy and cold seasons, which there were high volume of water, conductivities and TDS of both streams at all elevations were reduced with the dilution of rainwater. Since both streams are on the same mountain range with similar geological streambed then were there were not significantly different between streams.

There were few villages at the area between study sites of both streams at 1050 and 900 mAMSL which made pH increased along the streams probably from the household detergents which are base. Rain made the pH of both streams closely to 7 e.g., change pH 5 of Mae Sa Noi in dry season to around 7 in rainy and cold seasons. In 1999, rainy season was prolonged to cold season month (Table A8). Hence, the pH of two streams were non-significantly different in rainy and cold seasons at all elevations. By summary both streams at all elevations and seasons had pH around 5-7 which was in the normal range of natural water that allowed aquatic organisms to survive.

Although the statistical comparison of means showed the significantly different of velocity and pH between Mae Sa Noi and the control streams, but this difference was not occurred in cluster analysis. The cluster analysis clearly indicated seasonal changed of the physico-chemical parameters but did not clearly indicate the difference of the physico-chemical parameters of the two streams. The small groups of mixed stream sites were then grouped together at higher Square Euclidean Distance (SED), i.e. groups 1 and 3. In other words, the physico-chemical parameters of the two streams were similar at 3 SED. The differences of velocities and pH between the two streams (Table 4.2) caused the lower level of similarity (or higher value of SED)

between them. By summary both streams had comparable physico-chemical parameters to a certain extent.

5.2 Biological components

The highest population density and chironomid population density of both streams in all season was occurred at 900 mAMSL (Table 4.3 and 4.4). This elevation was located behind small waterfall. Then high velocity at this elevation flooded down the benthic macroinvertebrate to colonize at site 900 mAMSL where far from waterfall around 10 and 5 meters in Mae Sa Noi and the control streams, respectively. Although sites at 900 mAMSL were near waterfall, but water speed in these sites were not too high, they were closely with 700 mAMSL (Table 4.1 and 4.2). There was not significantly different of population density between seasons. The community had Diptera, Ephemeroptera and Trichoptera as major compositions. These macroinvertebrates especially Diptera lived in water continuously generation to generation all year round so that the population density did not changed seasonally. The population densities of Mae Sa Noi stream were significantly lower than the control stream which might be an impact from pesticides used in Mae Sa Noi stream and partly from the velocities.

Richness index, diversity index and evenness are indices for expression of community characteristics. Richness and diversity indices are indices showing abundant of species in community. In this study, at 1050 mAMSL had highest value of richness and diversity indices. This site had highest number of macroinvertebrate taxa. The value of these indices of Mae Sa Noi stream were higher than that of the control, so were the number of taxa present here. These 3 characteristics of

community; richness, diversity and evenness, have closely relation that in community having high richness and evenness the diversity will be high too. For example, at 1050 mAMSL of Mae Sa Noi stream in rainy season had high of richness and evenness which were 4.02 and 0.78 respectively then its diversity was 2.41. Whereas at 900 mAMSL of the control stream in cold season where had richness index 1.83 and evenness 0.58, then its diversity index was only 0.34. In other case, at 900 mAMSL of Mae Sa Noi stream in dry season had higher richness index than that of the community at 700 mAMSL of same stream and season but at the later had higher resulting in higher value of diversity index (Table 4.3).

Macroinvertebrate communities were grouped by stream (Fig 4.2). Since, Mae Sa Noi and the control streams had different of macroinvertebrate taxa (Tables A2-A4). Mae Sa Noi stream had a high number of simuliums while the control stream was dominated with chironomids. The reason of difference of the community between stream was agreed with the difference of velocity, but the level of pH though significantly different were in the range of natural water so may not have effective impact on the community.

The correlation between velocities and total population density showed positive significant correlations. At 900 mAMSL the high velocity encouraged abundant of macroinvertebrates, the total population and chironomid population densities. When this elevation was not considered, the correlation was non-significant. The range of current speed was not a limiting factor to chironomids' living in this elevation because chironomids are able to live in moderate or swift current stream (McCafferty, 1981).

Another physico-chemical parameter that was significantly different between streams was pH. The correlation between this parameter and total population density and chironomid population density was not significant. Moreover, in this study, the pH varied between 5-7 that was within normal of natural water pH which was not too stress for aquatic living-organisms to survive.

Mean comparison and cluster analysis of physico-chemical parameters showed the seasonal change. If only physico-chemical parameters rule the community characteristics, the population density and community should varied seasonally too. However, in the present research mean macroinvertebrate population density and their community were different between streams but not between seasons (Table 4.4 and Fig 4.2). This was confirmed by significant positive correlation between population densities and velocities but not pH. Hence, it can be postulated that there might be other factors influencing the change of population densities.

5.3 Chironomids ChE activity in Mae Sa Noi and the control stream

Most use pesticides in Ban Mae Sa Mai were OPs and CAs; Parathion (Folidol), Malathion (Malathions), Metamidophos (Tamaron), Methomyl (Lannate), Carbaryl (Sewin 85) (Stuetz, 1999). These pesticides had possibly contaminated in nearby stream; Mea Sa Noi stream, and caused impact to aquatic macroinvertebrate community. That in the present study found the significantly lower of macroinvertebrate population density than the control stream. Then the test ChE activity of chironomids was done for showing the impact of OPs and CAs to aquatic organisms using chironomids.

Chironomids were selected as a biomaker in this study because of many reasons i.e. they were abundant and found in all study site, then the sampling might be less to impact to ecological system. They were sedentary because chironomids always live in the sediment in area that full of liter. They were tolerance enough and quick recovery and there were more available literatures. But there were some disadvantages such as they were small in size (1-4 cm length), difficult to identify in to species and collect. However they were selected to use as biomarker in the present research.

The results showed chironimid ChE activities were reduced from up to downstream because of at the 1050 mAMSL were the areas that had not many disturbances while in the lower altitude areas (900 and 700 mAMSL) were probably contaminated with pesticides. Mae Sa Noi stream probably got pesticides directly from agricultural practice while the control stream might get some pesticides from aerial spray drifted from upper agricultural areas. Then there were found that ChE activities of chironomids in Mae Sa Noi stream were tendency lower than the control stream. ChE activities in chironomids were measured in rainy and cold seasons that it was found that in rainy season had higher ChE activity than in the cold season, because the influence of rain that diluted the concentration of pesticides contaminated in the stream. Besides, cold season was agricultural season since most vegetables were grown and while in rainy season fewer crops were grown.

ChE activity of chironomids sampled from Mae Sa Noi stream was lower than the control stream with non-significantly difference (Table 4.7). Therefore chironomid samples might be too small samples that were required for enough statistic power. Other reason, the contamination of OPs and CAs into stydy stream

might be quite low then their baseline ChE activities were slightly lower than from the control stream. However, this study was assured that ChE activity of chironomids sampled from Mae Sa Noi was disturbed from pesticides used in the surrounding area. Then the *in vitro* inhibition test was conducted

5.4 *In vitro* inhibition test

Methyl-parathion which was used in Mae Sa Mai agricultural area was extra added into chironomid samples from both streams. The increasing methyl-parathion concentrations into chironomid samples were caused increasing of % inhibition (100 - % of reduced ChE activities). Chironomids sampled from upstream (1050 mAMSL) from both streams were required higher concentration of methyl-parathion than chironomids sampled from lower elevation to increased % inhibition. Therefore the highest site of both streams were the area where less of disturbance from pesticides. Chironomids sampled from Mae Sa Noi stream had lower concentration of excess methyl-parathion causing % inhibition for 25, 50 and 75 percent than the control stream at same elevation. For example at 1050 mAMSL, 50% inhibition of chironomids ChE activity sampled from Mae Sa Noi stream was occurred at adding methyl-parathion to 135 μM while samples from the control must increased concentration to 225 μM for showing same inhibition percentage. These results agreed with the idea that ChE activity of chironomids from Mae Sa Noi were weaken than that from the control stream probably because of their exposure experience to pesticides. An obviously supported idea the highest rate of % inhibition increasing was occurred in chironomids sampled from Mae Sa Noi stream at 900 mAMSL where locate in the center of agricultural area. That in this site only 35 μM of methyl-

parathion was caused 25 % inhibition and only 175 μM of adding pesticides caused 75% inhibition. On the other hand, the lowest inhibition rate was happened in chironomids sampled from the control stream at 1050 mAMSL this site required highest concentration of methyl-parathion to increased % inhibition because they lived in rather clean condition then they remained ability to receive more dose of pesticides than the other sites.

The properties of dose responses were explained with the word "susceptibility". Chironomids that required low dose or concentration of methyl-parathion were called high susceptibility. Then chironomids sampled from Mae Sa Noi stream were more susceptible to methyl-parathion than that from the control.

However, in the *in vitro* inhibition test, rather high concentration of pesticide was used. Methyl-parathion concentration of 10 mM was prepared for getting 307 μM in testing condition. This could be explained that *in vitro* test, the methyl-parathion could not be changed to active form; more toxic, methyl-paraoxon which stronger ChE inhibitor. That paraoxon occurred in *in vivo* condition when other biochemical metabolisms are more active.

Another factor affected the total and chironomid population densities were pesticides used beside the stream velocity. From the comparison of mean, the mean benthic macroinvertebrate and chironomid population densities of Mae Sa Noi stream were significantly lower than those of the control; $p=0.0266$ and $p=0.0002$, respectively and the changing of benthic community characteristics were obvious. Also the mean ChE activities of chironomid samples collected from Mae Sa Noi stream were lower than those from the control but not significantly different ($p=0.07$). Moreover, chironomid samples from Mae Sa Noi stream had higher susceptibility

than those from the control, i.e., the amount of methyl-parathion amount required to inhibit 50% of chironomid ChE activity from the Mae Sa Noi and the control streams were 121.7±24.8 and 202.5±25.6 μM respectively. These toxicological tests explained the difference of mean total and chironomid population densities of the two streams. The stream with lower velocity, higher susceptibility and low chironomid ChE activity had lower population densities. Moreover, the cluster analysis shown in fig 4.3 and 4.4 confirmed the differences between the two streams studied when evaluated with ChE activity, susceptibility, and total population and chironomid population densities. Therefore, it can be deducted that stream velocity and pesticides used caused the reduction of the population densities and the changing of community characteristics.